PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
237.F		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 26327	10/12/1998	12/12/1997
Applicant		
GILEAD SCIENCES, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   CYCLOHEXENE CARBOXYLATES AS NEURAMINIDASE INHIBITORS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. ////

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/26327

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C233/52 C07C69/75 C07C69/757 A61K31/16 A61K31/215

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 16320 A (BIOTA SCIENT MANAGEMENT) 31 October 1991 cited in the application	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 1999	07.04.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/US 98/ 26327

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 40-42 are directed to a method of treatment of the human/ animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9116320 A	31-10-1991	AP 249 A	17-03-1993
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		AU 7533891 A	12-12-1991
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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C07D 309/22, 309/20, 335/02
C07D 211/78, 211/74, 211/72
C07D 211/70

A1

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PK 2896	19 October 1990 (19.10.90)	AU
PK 4537	11 February 1991 (11.02.91)	AU

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(72) Inventors; and

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(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.
With amended claims.

(54) Title: DERIVATIVES AND ANALOGUES OF 2-DEOXY-2,3-DIDEHYDRO-N-ACETYL NEURAMINIC ACID AND THEIR USE AS ANTIVIRAL AGENTS

(57) Abstract

Derivatives and analogues of 2-deoxy-2,3-didehydro-N-acetyl neuraminic acid, pharmaceutical formulations thereof, methods for their preparation and their use in the treatment of viral infections, in particular influenza, are described.

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"DERIVATIVES AND ANALOGUES OF 2-DEOXY-2,3-DIDEHYDRO-N-ACETYL NEURAMINIC ACID AND THEIR USE AS ANTIVIRAL AGENTS".

This invention relates to a new class of chemical compounds and to their use in medicine. In particular the invention concerns new 4-substituted-2-deoxy 2,3-didehydro derivatives of α-D-neuraminic acid, methods for their preparation, pharmaceutical formulations thereof and their use as antiviral agents.

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many microorganisms. These include bacteria such as Vibrio cholerae, Clostridium perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology 1974 58 457-63. The most active of these is 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses in vitro. See Palese et al., Virology 1974 59 490-498.

A number of 2-deoxy-2,3-didehydro-N-acetyl-neuraminic acid derivatives are known in the art. See for example P. Meindl et al., Virology, 58, 457-463 (1974); P. Meindl and H. Tuppy, Mh. Chem, 100 (4), 1295-1306 (1969); M. Flashner et al., Carbohydrate Research, 103, 281-285 (1982); E. Zbiral et al., Liebigs Ann Chem, 159-165 (1989); T. Ogawa

and Y. Ito, Tetrahedron Letters, 28 (49), 6221-6224 (1987); T. Goto et al., Tetrahedron letters, 27 (43), 5229-5232 (1986); H. Ogura et al., Chem. Pharm. Bull, 36 (12), 4807-4813 (1988); German Offenlegungschrift P 1439249. Many of these compounds are active in vitro against neuraminidase from V. cholerae or Newcastle disease virus as well as that from influenza virus. Neuraminidase in at least some strains of influenza or parainfluenza viruses has also been reported to be inhibited in vitro by 3-aza-2,3,4-trideoxy-4-oxo-D-arabinoctonic acid δ-lactone and O-α-N-acetyl-D-neuraminosyl-)2--->3)-2-acetamido-2-deoxy-D-glucose. See Zakstel'skaya et al., Vop. Virol. 1972 17 223-28.

Neuraminidase from Arthrobacter sialophilus is inhibited in vitro by the glycals 2,3-dehydro-4-epi-N-acetyl-neuraminic acid, 2,3-dehydro-2-deoxy-N-acetylneuraminic acid and 5-acetamido-2,6-anhydro-2,3,5-trideoxy-D-manno-non-2-en-4-ulosonate, and by their methyl esters. See Kumar et al., Carbohydrate Res. 1981 94 123-130; Carbohydrate Res. 1982 103 281-285. The thio analogues 2-α-azido-6-thio-neuraminic acid and 2-deoxy-2,3-didehydro-6-thioneuraminic acid, Mack & Brossmer, Tetrahedron Letters 1987 28 191-194, and the fluorinated analogue N-acetyl-2,3-difluoro-α-D-neuraminic acid, Nakajima et al., Agric. Biol. Chem. 1988 52 1209-1215, were reported to inhibit neuraminidase, although the type of neuraminidase was not identified. Schmid et al., Tetrahedron Letters 1958 29 3643-3646, described the synthesis of 2-deoxy-N-acetyl-α-D-neuraminic acid, but did not report its activity or otherwise against neuraminidase.

None of the known inhibitors of neuraminidase activity in vitro has been shown to possess antiviral activity in vivo, and indeed some, such as FANA, have specifically been shown to be inactive in vivo. Thus the conventional wisdom has accordingly considered that compounds exhibiting in vitro inhibition of viral neuraminidase would not effect an in vivo blockade of virus infection.

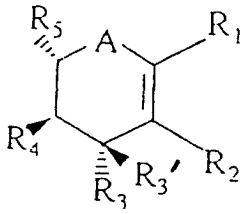
Meindl and Tuppy, Hoppe-Seyler's Z. Physiol Chem. 1969 350 1088, described hydrogenation of the olefinic double bond of 2-deoxy-2,3-dehydro-N-acetylneuraminic acid to

produce the β-anomer of 2-deoxy-N-acetylneuraminic acid. This β-anomer did not inhibit Vibrio cholerae neuraminidase.

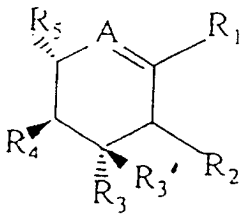
The most potent in vitro inhibitors of viral neuraminidase have thus been identified as compounds that are based on the neuraminic acid framework, and these are thought by some to be transition-state analogues. Miller et al., Biochem. Biophys. Res. Comm. 1978 83 1479. But while many of the aforementioned neuraminic acid analogues are competitive inhibitors of neuraminidases, to date, none has been reported as showing anti-viral activity in vivo. For example, although a half-planar, unsaturated 6-member ring system has been asserted to be important for inhibitory activity, see Dernick et al. in ANTIVIRAL CHEMOTHERAPY (K. K. Gauri ed.) Academic Press, 1981, at pages 327-336, some compounds characterized by such a system, notably FANA, have been reported not to possess in vivo anti-viral activity. See Palese and Schulman in CHEMOPROPHYLAXIS AND VIRUS INFECTION OF THE UPPER RESPIRATORY TRACT, Vol. 1 (J. S. Oxford ed.) CRC Press, 1977, at pages 189-205.

We have now found novel 4-substituted 2-deoxy-2,3-didehydro derivatives of α-D-neuraminic acid which are active in vivo.

The invention therefore provides in a first aspect compounds of formula (I) or formula (Ia)



(I)



(Ia)

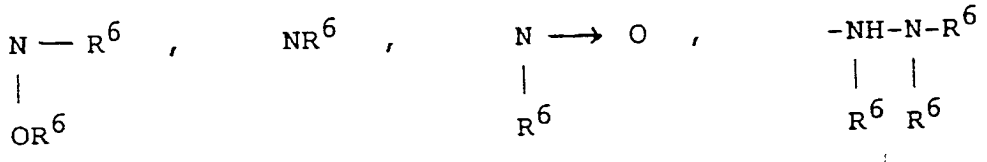
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

R^1 denotes COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, CHO or $CH(CHO)_2$,

R^2 denotes H, OR^6, F, Cl, Br, CN, NHR^6, SR^6 or CH_2X, wherein X is NHR^6, halogen or OR^6 and

R^6 is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group,

R^3 and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, NHR^6, N_3, SR^6, $=N-OR^6$, OR^6, guanidino,



R^4 denotes NHR^6, SR^6, OR^6, $COOR^6$, NO_2, $C(R^6)_3$, CH_2COOR^6, CH_2NO_2 or CH_2NHR^6, and

R^5 denotes CH_2YR^6, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an R^5 group are the same or different,

and pharmaceutically acceptable salts or derivatives thereof.

In both these formulae R^1, R^2, R^3, $R^{3'}$, R^4, R^5 and R^6 are subject to the provisos that in general formula (I),

(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

(a) an R^2 that is hydrogen and

(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen, and that in general formula (Ia),

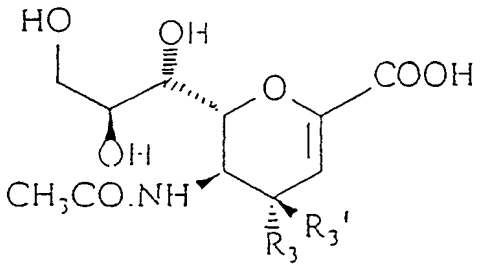
(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an R^2 that is hydrogen, and

(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen.

In a preferred embodiment, the compound has general formula (II)



(II)

i.e. in general formula (I) above, R^1 is COOH, R^2 is hydrogen, R^4 is acetamido, and R^5 is $-CHOH.CHOH.CH_2OH$, and R^3 is hydrogen or $R^{3'}$, where $R^{3'}$ denotes $-N_3$, $-CN$, $-CH_2NH_2$, or $-N.R^8.R^9$;

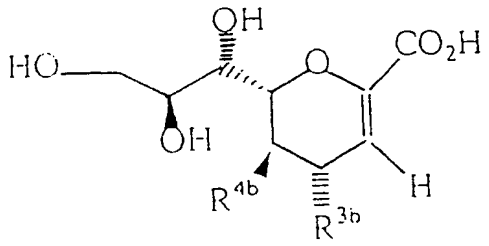
R^8 and R^9 are the same or different, and each denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substitued acyl group of 1 to 6 carbon atoms, $-C.(NH).NH_2$, $-CH_2.COOH$, $-CH_2CH_2-OH$ or $-CH_2.CH.(R^{10})(R^{11})$,

R^{10} and R^{11} may be the same or different, and each denotes oxygen or $R^{12}N=$, and

R^{12} denotes hydrogen, $-OH$, $-OCH_3$, $-NH_2$, or $(CH_3)_2N-$.

We have found a particular subclass of compounds of formula (I) which are unexpectedly more active than their corresponding 4-hydroxy analogues.

Thus in a particularly preferred aspect the invention provides compounds of formula (Ib)



(Ib)

wherein R^{3b} is $(alk)_xNR^{6b}R^{7b}$, CN or N_3

where alk is unsubstituted or substituted methylene,

x is 0 or 1

R^{6b} is hydrogen, C_{1-6}alkyl (e.g. methyl, ethyl), aryl (e.g. phenyl), aralkyl (e.g. phenC_{1-4}alkyl such as benzyl), amidine, $NR^{7b}R^{8b}$, or an unsaturated or saturated ring containing one or more heteroatoms (such as nitrogen, oxygen or sulphur),

R^{7b} is hydrogen, C_{1-6}alkyl (e.g. methyl, ethyl), or allyl, or $NR^{6b}R^{7b}$ forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms (such as nitrogen, oxygen or sulphur), R^{8b} is hydrogen or C_{1-6}alkyl, and

R^{4b} is $NHCOR^{9b}$ where R^{9b} is hydrogen, substituted or unsubstituted C_{1-4}alkyl or aryl,

and pharmaceutically acceptable salts of the compounds of formula (Ib) and their pharmaceutically acceptable derivatives.

In the compounds of formula (Ib) the substituents (for example the group R^6 in the substituent R^3) may themselves bear substituents conventionally associated in the art of pharmaceutical chemistry with such substituents.

Preferably R^3 is NR^6R^7, in particular NH_2 or guanidino.

Preferably R^4 is $NHCOR^9$ where R^9 is methyl or halogen substituted methyl (e.g. FCH_2, F_2CH-, F_3C).

References herein to preferred definitions of groups in compounds of formula (I) apply <u>mutatis mutandis</u> to the corresponding groups in formulae (Ia), (Ib) and (II).

C_{1-4}alkyl as used herein includes both straight chain (e.g. methyl, ethyl) and branched chain (e.g.

isopropyl, t-butyl) alkyl groups.

By pharmaceutically acceptable derivative is meant any pharmaceutically acceptable ester or salt of such ester of the compounds of formula (I) or any other compound which upon administration to the recipient is capable of providing (directly or indirectly) a compound of formula (I) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compounds. Of particular interest as such derivatives are compounds modified at the C-1 carboxyl function, the C-7 or C-9 hydroxyl functions, or at amino groups. Thus compounds of interest include C_{1-4}alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzoyl) esters of the compounds of formula (I), C-7 or C-9 esters of compounds of formula (I) such as acetyl esters thereof, C-7 or C-9 ethers such as phenyl ethers, benzyl ethers, p-tolyl ethers, and acylated amino derivatives such as formyl, acetamido.

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compounds of formula (I) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable, inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali

metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4}alkyl) salts.

References hereinafter to a compound of the invention include the compounds of formula (I) and pharmaceutically acceptable salts and derivatives thereof.

Particularly preferred compounds of the invention include :-

5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (also known as 5-(acetylamino)-4-amino-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid), salts thereof including the sodium salt and 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (also known as 5-(Acetylamino)-2,6-anhydro-4-guanidino-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid) and salts thereof, including the ammonium salt.

The compounds of formula (I) possess antiviral activity. In particular these compounds are inhibitors of viral neuraminidase of orthomyxoviruses and paramyxoviruses for example the viral neuraminidase of influenza A and B, parainfluenza, mumps, and Newcastle disease, fowl plague and Sendai virus.

There is thus provided in a further aspect of the invention a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof for use as an active therapeutic agent, in particular as an antiviral agent, for example in the treatment of orthomyxovirus and paramyxovirus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections in a mammal including man, comprising the step of administering to said mammal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

There is also provided in a further or alternative aspect use of a compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art

that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of a compound of the invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient, and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, a suitable dose will be in the range of from about 0.01 to 750mg/kg of bodyweight per day preferably in the range of 0.1 to 100 mg/kg/day, most preferably in the range of 0.5 to 25 mg/kg/day.

In particular we have found that the effective doses of the compounds tested are related to their in vitro potency. Thus DANA (which has IC_{50} plaque reduction of 5µg/ml) has been found to be effective at doses of between 1 and 10mg/kg per treatment. The corresponding methyl ester of DANA (IC_{50} 50-100µg/ml) is effective at proportionally higher dose.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compounds are also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7, e.g. 5 days post infection depending upon the particular compound used.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage from for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, a compound of the invention may be administered as the raw chemical, it is preferable to present the active ingredient

as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising a compound of the formula (I) or formula (Ia), but not subject to the proviso thereto, or a pharmaceutically acceptable salt or derivative thereof together with a pharmaceutically acceptable carrier therefor.

The carrier must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The pharmaceutical formulations may be in the form of conventional formulations for the intended mode of administration.

For intranasal administration according to the method of the invention the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for intranasal administration.

Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous, for example prepared from water alone (for example sterile or pyrogen-free water), or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol, and polyethylene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (e.g. Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a

dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Intranasal administration may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluroroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form, for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In the intranasal formulations the compound will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired the formulations may be adapted to give sustained release of the active ingredient. The compounds of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compounds of the invention may be employed with other antiviral agents. The invention thus provides in a further aspect a combination comprising a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof together with another

therapeutically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

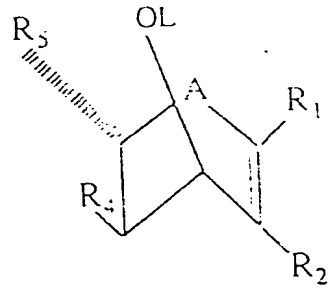
Suitable therapeutic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds effective against influenza viruses, such as amantadine, rimantadine and ribavirin, may be included in such combinations.

The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compounds of the invention are used with a second therapeutic agent active against the same virus, the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compound of formula (I) and its pharmaceutically acceptable salts and derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure.

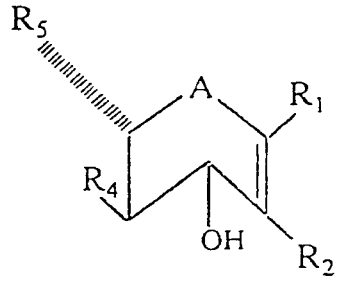
In one such process (A) a compound of formula (III)



(III)

wherein R^2 is as defined in formula (I), and L is a leaving group (for example a sulphonic acid residue such as tosyl, mesyl, trifluoromesyl) or a protected derivative thereof is reacted with the appropriate nucleophile, for example azide, cyanide, an appropriate carbanion, or thioacetate.

The compounds of formula (III) may be obtained from the corresponding compounds of formula (IV)



(IV)

by inversion of the 4-OH group by methods known in the art, for example by reaction with a Lewis acid (such as BF_3 etherate) followed by hydrolysis. The compounds of formula (IV) are either known in the art or may be obtained by methods analogous to those for preparing the known compounds.

In a second method (B) the compounds of formula (I) may be prepared from other compounds of formula (I) by interconversion. Thus compounds of formula (I) wherein R^3 is NH_2 or CH_2NH_2 may be prepared by reduction of the corresponding azido or cyano analogues respectively.

Compounds wherein R^3 is NH alkyl or guanidino may be prepared by derivatisation of the corresponding compound wherein R^3 is NH_2.

Compounds of formula I where R^1 is COOH may be prepared by hydrolysis of the corresponding ester under either acidic or basic conditions, for example at pH 11-12 (using a base such as sodium or ammonium hydroxide), or at pH 2-3 (using an acid such as sulphuric acid).

As will be appreciated by those skilled in the art, it may be necessary or desirable at any stage in the above described processes to protect one or more sensitive groups in the molecule to prevent undesirable side reactions; the protecting group may be removed at any convenient subsequent stage in the reaction sequence.

The protecting groups used in the preparation of compounds of formula (I) may be used in conventional manner. See for example 'Protective Groups in Organic Chemistry' Ed. J. F. W. McOmie (Plenum Press 1973) or 'Protective Groups in Organic Synthesis' by Theodora W Greene (John Wiley and Sons 1981).

Conventional amino protecting groups may include for example aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; and acyl groups such as N-benzyloxycarbonyl or t-butoxycarbonyl. Thus, compounds of general formula (I) wherein one or both of the groups R^2 and R^3 represent hydrogen may be prepared by deprotection of a corresponding protected compound.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, acyl groups, such as acetyl; silicon protecting groups, such as trimethylsilyl groups; or as tetrahydropyran derivatives.

Removal of any protecting groups present may be achieved by conventional procedures. Thus an aralkyl group, such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst (e.g. palladium on charcoal); an acyl group such as N-benzyloxycarbonyl, may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid or by reduction, for example by catalytic hydrogenation; silicon protecting groups may be removed, for example, by treatment with fluoride ion; tetrahydropyran groups may be cleaved by hydrolysis under acidic conditions.

Where it is desired to isolate a compound of the invention as a salt, for example as an acid addition salt, this may be achieved by treating the free base of general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

The present invention is further described by the following examples, which are for illustrative purposes only, and should not be construed as a limitation of the invention.

General Methodologies

The following general methods are appliicable to the synthesis of compounds of the invention.

Deacetylation

Treatment of the acetylated material with Amberlite IRA-400 (OH^-) with stirring, for a period of time, generally 2-3 h, at room temperature results in complete de-O-acetylation. The resin is filtered off and the filtrate concentrated to dryness to afford the desired de-O-acetylation material.

Those skilled in the art would recognise that other standard procedures are available for the complete de-O-acetylation of the same material, such as treatment with sodium methoxide in methanol.

Deesterification

The completely de-O-acetylated material is taken up in aqueous sodium hydroxide and stirred at room temperature for a period of time, generally 2-3 h. The mixture is then adjusted to pH 7.0-7.5 with Dowex 50w X 8 (H^+) resin. Filtration followed by freeze-drying of the filtrate affords the desired deesterified material.

Those skilled in the art would readily be able to identify several alternative options for the deesterification of the same material such as acid hydrolysis, alternative base hydrolyses e.g. ammonium hydroxide, potassium hydroxide.

Intermediate compounds referred to in Examples 1 to 15 are identified as follows:

COMPOUND 2

Methyl 5-acetamido-7,8,9-tri-O-acetyl-2,3,5-trideoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 3

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-azido-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 5

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 8

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-diallylamino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 10

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-allylamino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 12

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-4-aminoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 13

Methyl 7,8,9-tri-O-acetyl-2,3,5-trideoxy-4',5'-dihydro-2'-methyloxazolo [5,4-d] D-glycero-D-talo-non-2-enopyranosonate (4-epi-4,5-oxazaloNeu7,8,9$Ac_3$2en1Me)

COMPOUND 15

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 16

Methyl 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5Ac2en1Me)

COMPOUND 18

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-methylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methylamino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 19

Methyl 5-acetamido-4-N-methylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methylamino-Neu5Ac2en1Me)

COMPOUND 21

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2enopyranosonate (4-N,N-dimethylamino-Neu5,7,8,9$Ac_4$2en1Me)

COMPOUND 22

Methyl 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-dimethylaminoNeu5Ac2en1Me)

COMPOUND 24

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-methoxycarbonylmethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methoxycarbonylmethylaminoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 25

Methyl 5-acetamido-4-N-methoxycarbonylmethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-methoxycarbonylmethylaminoNeu5Ac2en1Me)

COMPOUND 27

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5,7,8,9-$Ac_4$2en1Me)

COMPOUND 28

Methyl 5-acetamido-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5,7,8,9$Ac_4$2en1Me)

COMPOUND 29

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-2′-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-2′-hydroxyethylaminoNeu5Ac2en1Me)

COMPOUND 30

3-Deoxy-D-glycero-D-galacto-2-nonulopyranosonic acid (KDN)

COMPOUND 31

Methyl 3-Deoxy-D-glycero-D-galacto-2-nonulopyranosonate (KDN1Me)

COMPOUND 32

Methyl (4,5,7,8,9-penta-O-acetyl-2,3-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl chlorid)onate (KDN4,5,7,8,9$Ac_5$2βCl1Me)

COMPOUND 33

Methyl 4,5,7,8,9-penta-O-acetyl-2,3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate (KDN4,5,7,8,9$Ac_5$2en1Me)

COMPOUND 34

Methyl 2,3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate (KDN2en1Me)

COMPOUND 36

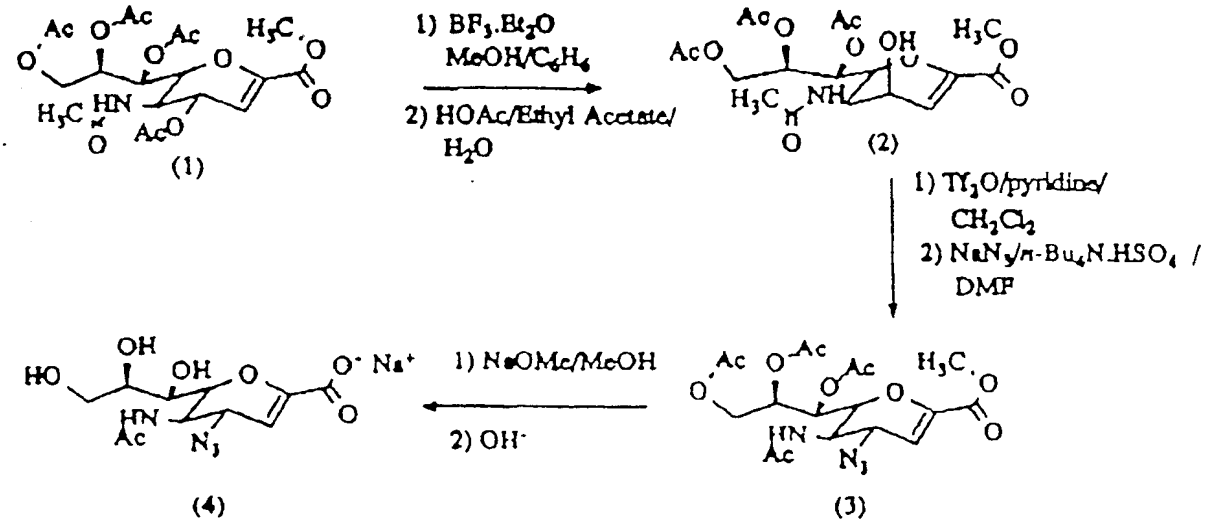
Hydrazinium 4,5-diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (Hydrazinium 4,5-diaminoNeu2en)

COMPOUND 37

4,5-diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (4,5-diaminoNeu2en)

Example 1 The preparation of Sodium 5-Acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-Azido-Neu5Ac2en) (4)

The overall reaction scheme is as follows:



Preparation of (2)

To an agitated solution of methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (1) (1500 mg, 3.17 mmol) in a mixture of benzene (50 ml) and methanol (300 mg) was added dropwise BF_3Et_2O (12 ml) over thirty minutes under a nitrogen atmosphere at room temperature. The whole mixture was then allowed to stir at room temperature for 16 hours. The solution was diluted with ethyl acetate (250 ml), washed successively with saturated $NaHCO_3$ solution (30 ml x 3) and water (20 ml x 3), then evaporated to a small volume (about 10 ml), to which was added water (0.5 ml) and acetic acid (0.5 ml). The whole mixture was then stirred at room temperature for two days before being diluted with ethyl acetate (200 ml). The ethyl acetate solution was washed with 5% $NaHCO_3$ solution (30 ml x 2) and water (20 ml x 3), then evaporated to dryness. The residue was chromatographed (silica gel, ethyl acetate as eluting solvent) to afford pure compound (2) (550 mg, 40%).

^{1}H-nmr ($CDCl_3$) δ (ppm); 1.95, 2.06, 2.08, 2.10, 2.35 (s, 15H, Acetyl CH_3 x 5), 3.80 (s, 3H, $COOCH_3$), 4.1-4.4 (m, 4H, H_4, H_5, H_6, H_9), 4.82 (dd, 1H, $J_{9,8}$ 1.8Hz, $J_{9,9}$, 12.3Hz, H_9), 5.27 (m, 1H, H_8), 5.45 (dd, 1H, $J_{7,8}$ 3.5Hz, H_7), 6.15 (d, 1H, $J_{3,4}$ 5.4Hz, H_3), 6.47 (d, 1H, $J_{NH,5}$ 8.8Hz, -CONH).

Preparation of (3)

To a stirred solution of compound (2) (800 mg, 1.67 mmol) in anhydrous dichloromethane (10 ml) and dry pyridine (316 mg, 4 mmol) at -30° to -40°C, was added dropwise a solution of trifluoromethane sulphonic anhydride (Tf_2O) (556 mg, 2 mmol) in dichloromethane (2 ml) over 15 minutes. The reaction mixture was then stirred at -30° for 5 hours, and concentrated to dryness in vacuo. The residue was then dissolved in dry DMF (5 ml) containing a mixture of sodium azide (650 mg, 10 mmol) and tetrabutylammonium hydrogen sulphate (170 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 16 hours, and then evaporated

to dryness under high vacuum. The residue was partitioned between ethyl acetate (200 ml) and water (50 ml). The organic layer was separated and washed with water (50 ml x 2), dried over Na_2SO_4, evaporated to leave a residue (780 mg), which was subjected to double chromatography (silica gel, the first solvent system was ethyl acetate/acetone: 8/1; the second solvent system was dichloromethane/water: 10/1) to afford a colourless oil (3) (185 mg, 24%).

MS. (FAB) 457 ($M^+ + 1$), 414 ($M^+ - N_3$. $[\alpha]^{20}_D$ + 19.1° (Cl,MeOH).ir.($CHCl_3$) cm^{-1} 2100 (N-N_3). 1748 (carbonyl). ^{1}H-nmr ($CDCl_3$) δ (ppm). 2.04, 2.05, 2.06, 2.12, (s, 12H, Acetyl CH_3 x 4). 3.79 (s, 3H, $COOCH_3$), 3.91 (ddd, 1H, $J_{5,NH}$ 8.4Hz, $J_{5,4}$ 8.8 Hz, $J_{5,6}$ 9.9Hz, H_5), 4.17 (dd, 1H, $J_{9',8}$ 6.8Hz, $J_{9',9}$, 12.5Hz, $H_{8'}$), 4.42 (dd, 1H, $J_{4,3}$ 2.9Hz, $J_{4,5}$ 8.8Hz, H_4), 4.48 (dd, 1H, $J_{6,7}$ 2.3Hz, $J_{6,5}$ 9.9Hz, H_6 4.46 (dd, 1H, $J_{9,8}$ 2.7Hz, $J_{9,9'}$ 12.5Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 5.2Hz, $J_{8,9}$ 2.7Hz, $J_{8,9'}$, 6.8Hz, H_8), 5.45 (dd, 1H, $J_{7,6}$ 2.3Hz, $J_{7,8}$ 5.2Hz, H_7), 5.96 (d, 1H, $J_{3,4}$ 2.9H, H_3), 6.13 (d, 1H, $J_{NH,5}$ 8.4Hz, -CONH) ^{13}C-$_{nmr}$ ($CDCl_3$) δ (ppm)

20.7 (CH_3-CO-O-), 23.2 (CH_3CO-NH), 48.3 (C_5), 52.6 ($COOCH_3$), 57.8 (C_4), 62.1 (C_9), 67.7, 70.9 (C_7, C_8), 75.9 (C_6), 107.6 (C_3), 145.1 (C_2), 161.5 (C_1), 170.2, 170.3, 170.7, (acetyl -C = 0 x 4).

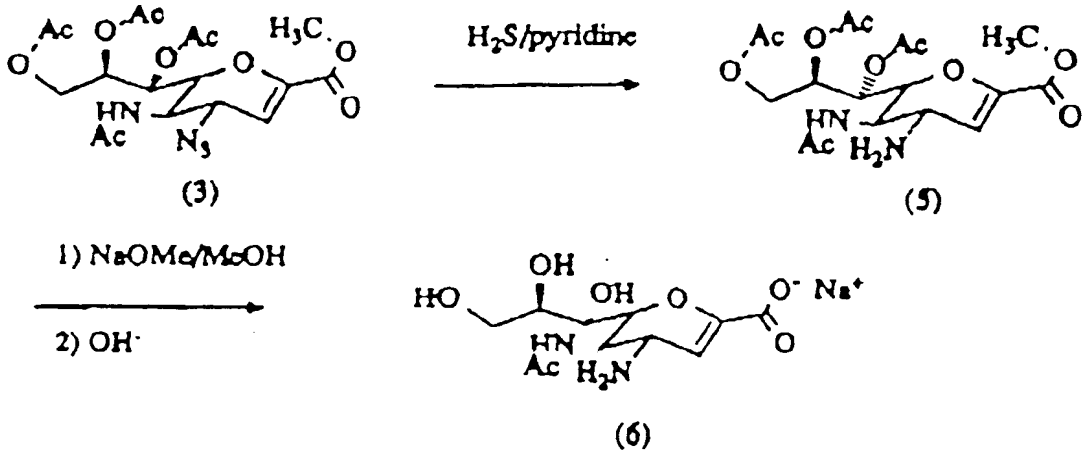
Preparation of (4)

Compound (3) (50 mg, 0.11 mmol) was dissolved in anhydrous methanol (5 ml) containing sodium methoxide (8 mg, 0.15 mmol). The mixture was stirred at room temperature for 2 hours and concentrated to dryness in vacuo. The residue was taken up in water (3 ml), stirred at room temperature for 1.5 hours, ajusted to pH 6-7 with Dowex 50 x 8 (H^+) resin, and then lyophilised to afford the title compound (4) (35 mg, 94%).

i.r. (KBr)cm^{-1} 3400 (br.-OH), 2100 ($-N_3$), 1714 (carbonyl). ^{1}H-nmr (D_2O) δ (ppm). 2.06 (5, 3H, acetyl CH_3), 3.64 (dd, 1H, $J_{9',8}$ 6.3Hz, $J_{9',9}$ 11.8Hz, $H_{9'}$), 3.65 (dd, 1H, $J_{7,6}$ 3.9Hz, $J_{7.8}$ 6.8Hz, H_7), 3.88 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 11.8Hz, H_9), 3.94 (m, 1H, $J_{8,7}$ 6.8Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 6.3Hz, H_8), 4.21 (dd, 1H, $J_{5,4}$ 10.4Hz, $J_{5,6}$ 8.9Hz, H_5), 4.31 (dd, 1H, $J_{4,3}$ 2.2Hz, $J_{4,5}$ 2.2Hz, $J_{4,5}$ 10.4Hz, H_4), 4.34 (dd, 1H, $J_{6,5}$ 8.9Hz, $J_{6,7}$ 3.9Hz, H_6) 5.82 (d, 1H, $J_{3,4}$ 2.2Hz, H_3).

Example 2 The preparation of Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6)

The overall reaction scheme is as follows:



(3) (5) (6)

Preparation of (5)

Into a solution of methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (3) prepared as in Example 1, (95 mg, 0.208 mmol) in pyridine (6 ml) was bubbled with H_2S for 16 hours at room temperature. The solution was then flushed with nitrogen for 15 minutes, and evaporated to remove pyridine under high vacuum. The residue was chromatographed (silica gel, ethyl acetate/isopropanol/water = 5/2/1) to afford a colourless compound (5) (50 mg, 56%).

MS. (FAB) 431 ($M^+ + 1$), 414 ($M^+ -NH_2$), $[\alpha]^{20}_D$ +34.5° (Cl, MeOH). i.r. ($CHCl_3$) cm^{-1} 3400 (br.NH_2), 1740 (carbonyl). ^{1}H-nmr ($CDCl_3$ + CD_3OD) δ (ppm). 1.96, 2.06, 2.07, 2.10 (s, 12H acetyl CH_3 x 4), 3.81 (S, 3H, $-COOCH_3$), 3.92 (brt, 1H, $J_{5,4}$ & $J_{5,6}$ 10Hz, H_5), 4.17 (dd, 1H, $J_{9',8}$ 7.2Hz, $J_{9',9}$ 12.3Hz, $H_{9'}$), 4.22 (br. dd, 2H, $J_{4,5}$ & $J_{6,5}$ 10Hz, $J_{4,3}$ & $J_{6,7}$ 2.1Hz, H_4 & H_6), 4.71 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 4.9Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 7.2Hz, H_8), 5.45 (d, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.9Hz, H_7), 5.97 (d, 1H, $J_{3,4}$ 2.1Hz, H_3).

^{13}C-nmr ($CDCl_3$ + CD_3OD) δ (ppm)

20.2, 20.3 (CH_3-CO-O-), 22.3 (CH_3-CO-NH), 48.2 (C_5), 50.4 (C_4), 52.0 ($COOCH_3$), 52.1 (C_9), 67.8, 71.2 (C_7, C_8), 76.5 (C_6), 112.5 (C_3), 143.5 (C_2), 162.0 (C_1), 170.2, 170.4, 170.8, 172.2 (acetyl -C = 0 x 4).

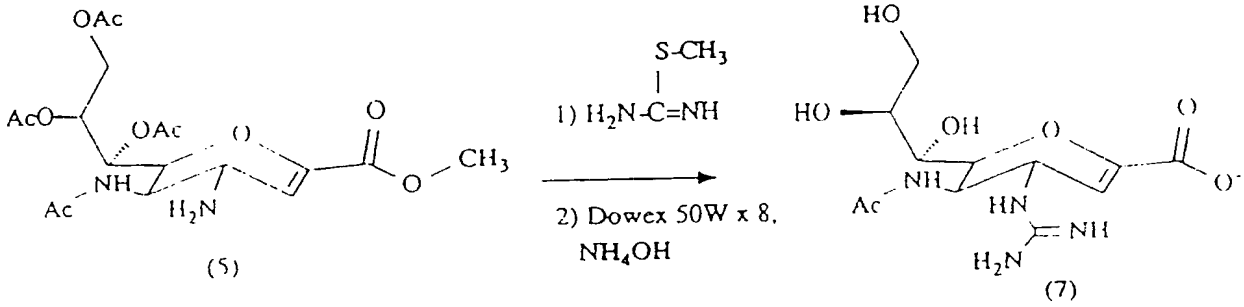
Preparation of (6)

Compound (5) (50 mg, 0.116 mmol) was dissolved in anhydrous methanol (5 ml) containing sodium methoxide (12.4 mg, 0.23 mmol). The mixture was stirred at room temperature for 1.5 hours and evaporated to dryness in vacuo at 30°C. The residue was stirred in water (3 ml) at room temperature until TLC (silica gel, ethyl acetate/methanol/0.1 N HCl = 5/4/1) indicated that hydrolysis was complete. The solution (pH about 10.5) was then gradually adjusted to around pH 7.5 by Dowex 50 x 8 (H^+) resin. As soon as the pH of the solution reached 7.5, the suspension was quickly filtered by a press filter. The filtrate was lyophilised to afford the title compound (6) (30 mg, 83%).

^{1}H-nmr (D_2O) δ (ppm). 2.07 (S, 3H, acetyl CH_3), 3.59 - 3.70 m, 2H, H_7 & $H_{9'}$), 3.89 (dd, 1H $J_{9,8}$ 2.6Hz, $-J_{9,9'}$ 11.8Hz, H_9), 3.95 (m, 1H, H_8), 3.99 (brd, 1H, $J_{4,5}$ 10.6Hz, H_4), 4.21 (brt, 1H, $J_{5,4}$ & $J_{5,6}$ 10.6Hz, H_5), 4.29 (brd, 1H, $J_{6,5}$ 10.6Hz, H_6), 5.66 (d, 1H $J_{3,4}$ 1.9Hz, H_3).

Example 3 The preparation of Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7)

The overall reaction scheme is as follows:



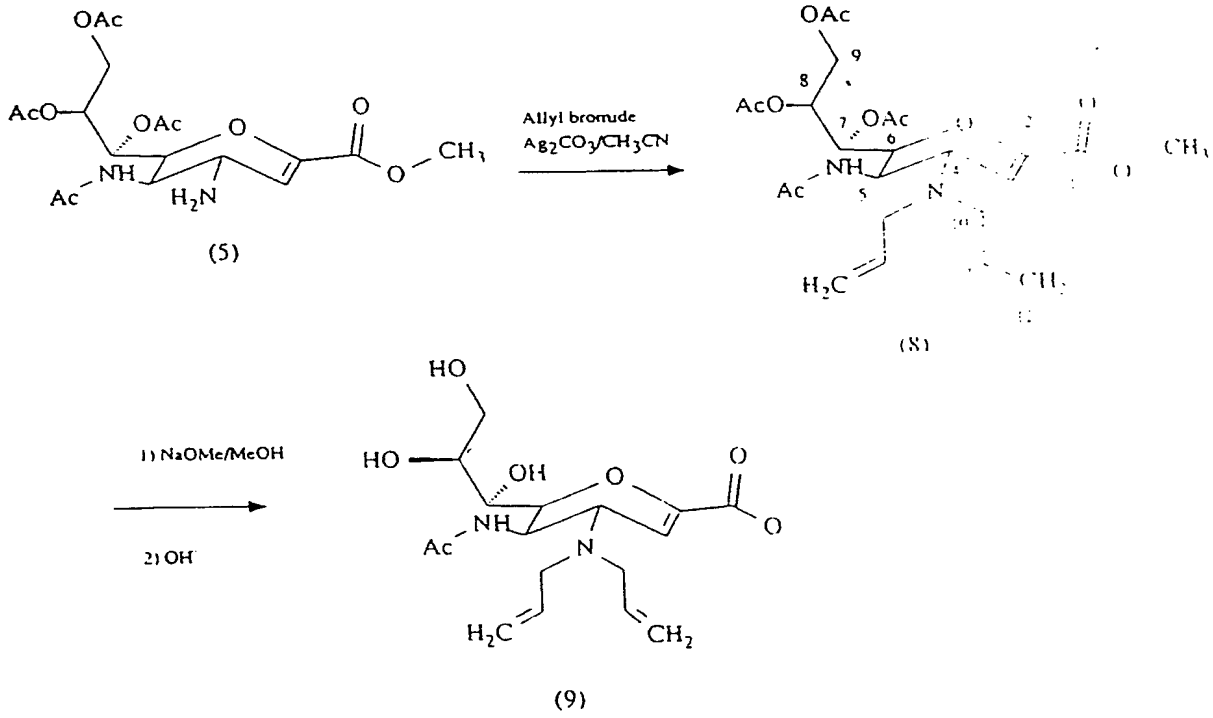
Into a solution of S-methylisourea (546 mg, 3 mmol) in water (15 mL) at ice-bath temperature, methyl-5,7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (5) prepared as in Example 2 (40 mg, 0.093 mmol) was added. The reaction mixture was stirred at 5°C for seven days and poured onto a column of Dowex 50W X 8 (H^+) resin (35 mL). The column was then washed with cold water (700 mL) and eluted with 1.5 M NH_4OH solution. The eluate (120 mL) was concentrated to dryness under high vacuum. The resulting residue was chromatographed (silica gel; solvent system 1: ethyl acetate/isopropanol/water, 1/5/1; solvent system 2: 75% isopropanol) to provide the title compound (7) (8 mg, 24.5%).

Compound (7) gave a strong, positive Sakaguchi reaction, indicating the presence of a guanidine group. NMR data for compound (7) are given below. ^{1}H-nmr (D_2O + CD_3OD) δ (ppm).

2.06 (s, 2H, acetyl CH_3), 3.60 (br. d., 1H, $J_{7,8}$ 9.4Hz, H_7), 3.63 (dd, 1H, $J_{9',8}$ 6.2Hz, $J_{9',9}$ 11.8Hz, $H_{9'}$), 3.76 (br. d., 1H, $J_{4,5}$ 9.4Hz, H_4), 3.87 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$, 11.8Hz, H_9), 3.93 (ddd, 1H, $J_{8,7}$ 9.4Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 6.2Hz, H_8), 4.01 (dd, 1H, $J_{5,4}$ 9.4Hz, $J_{5,6}$ 10.6Hz, H_5), 4.20 (br. d., 1H $J_{6,5}$ 10.6Hz, H_6), 5.63 (d, 1H, $J_{3,4}$ 2.1Hz, H_3).

Example 4 Sodium 5-Acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate. (9).

The overall reaction scheme is as follows:



Into a solution of allyl bromide (60mg, 0.5mmol)

and methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (5) (90mg, 0.209mmol) in acetonitrile (5mL), was added silver carbonate (116mg, 0.418mmol). The mixture was stirred and protected from light at room temperature for 16 h. The resulting suspension was filtered, and the filtrate was evaporated to dryness. The residue was subjected to flash-column chromotography silica gel, ethyl acetate containing 10% methanol) to afford methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (8) (85mg, 80%).

^{1}H-nmr ($CDCl_3$) δ (ppm) 1.94, 2.05, 2.06, 2.11 (s, 12H, acetyl CH_3 x 4), 2.97 (dd, 2H, $J_{10a,10b}$ & $J_{10'a,10'b}$ 14.3Hz, $J_{10a,11}$ & $J_{10'a,11'}$ 7.6Hz, H_{10a} & $H_{10'a}$), 3.24 (dd, 2H, $J_{10b,10a}$ & $J_{10'b,10'a}$ 14.3Hz, $J_{10b,11}$ & $J_{10'b,11'}$ 4.9Hz, H_{10b} & $H_{10'b}$), 3.58 (dd, 1H, $J_{4,3}$ 2.4Hz, $J_{4,5}$ 9.3Hz, H_4), 3.79 (s, 3H, $COOCH_3$), 4.12-4.26 (m, 3H, H_6, $H_{9'}$, H_5), 4.70 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.09 (dd, 2H, $J_{12cis,11}$ & $J_{12'cis,11'}$ 10.6Hz, J_{12gem} & $J_{12'gem}$~1.5Hz, H_{12cis} & $H_{12'cis}$), 5.14 (dd, 2H, $J_{12trans,11}$ & $J_{12'trans,11'}$ 17.7Hz, J_{12gem} & $J_{12'gem}$ ~1.5Hz, $H_{12trans}$ & $H_{12'trans}$), 5.27-5.32 (m, 2H, H_8 & -CONH-), 5.55 (dd, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.7Hz, H_7), 5.72 (m, 2H, H_{11} & $H_{11'}$), 6.07 (d, 1H, $J_{3,4}$ 2.4Hz, H_3).

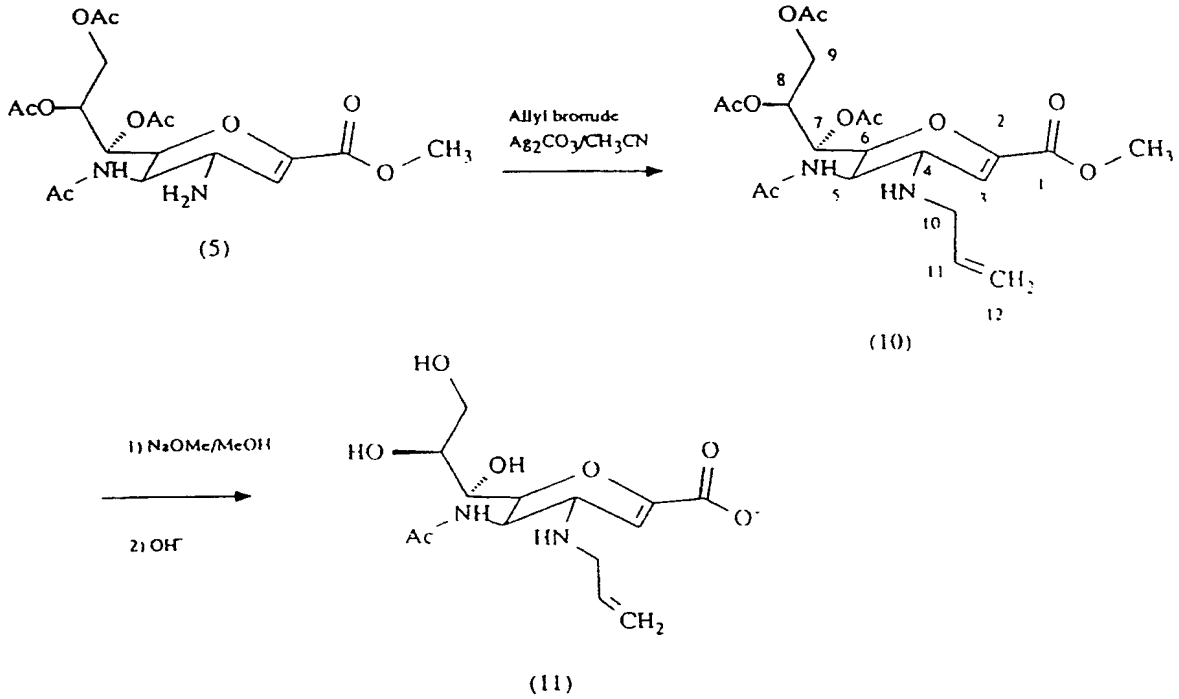
Compound (8) (80mg, 0.156mmol) was dissolved in anhydrous methanol (10mL) containing sodium methoxide (16.2mg, 0.30mmol).

The solution was stirred at room temperature for 2 h, then evaporated to dryness. The residue was taken up in water (5mL), and left at room temperature for 2 h. The resulting solution was neutralized with Dowex 50 x 8 (H^+) and freeze-dried to afford the title compound (9) (49mg, 80%).

^{1}H-nmr (D2O) δ (ppm) 1.94 (s, 3H, Acetyl CH_3), 3.24-3.44 (m, 4H, H_{10} x 2 & $H_{10'}$ x 2), 3.48-4.33 (m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.24-5.29 (m, 4H, H_{12} x 2 & $H_{12'}$ x 2), 5.69 (d, 1H, $J_{3,4}$ ~2Hz, H_3), 5.73-5.76 (m, 2H, H_{11} & $H_{11'}$)

Example 5 Sodium 5-Acetamido-4-N-allylamino-2,3,4,5-tetra-deoxy-D-glycero-D-galacto-non-2-enopyranosonate (11)

The overall reaction scheme was as follows:



To a solution of allyl bromide (48mg, 0.40mmol) and compound (5) (155mg, 0.36mmol) in acetonitrile (5mL) was added silver carbonate (107mg, 0.38mmol). The mixture was stirred, whilst protected from light, at room temperature for 16 h. The resulting suspension was filtered off, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (ethyl acetate/ isopropanol/water = 5:2:1). Fractions with an Rf value of 0.5 were combined and evaporated to dryness to afford compound (10) (53mg, 32%). The starting material (5) with an Rf value of 0.3 (61mg, 39%) and N, N-diallyl derivative (8) with an Rf value of 0.9 (20mg, 11%) were recovered respectively.

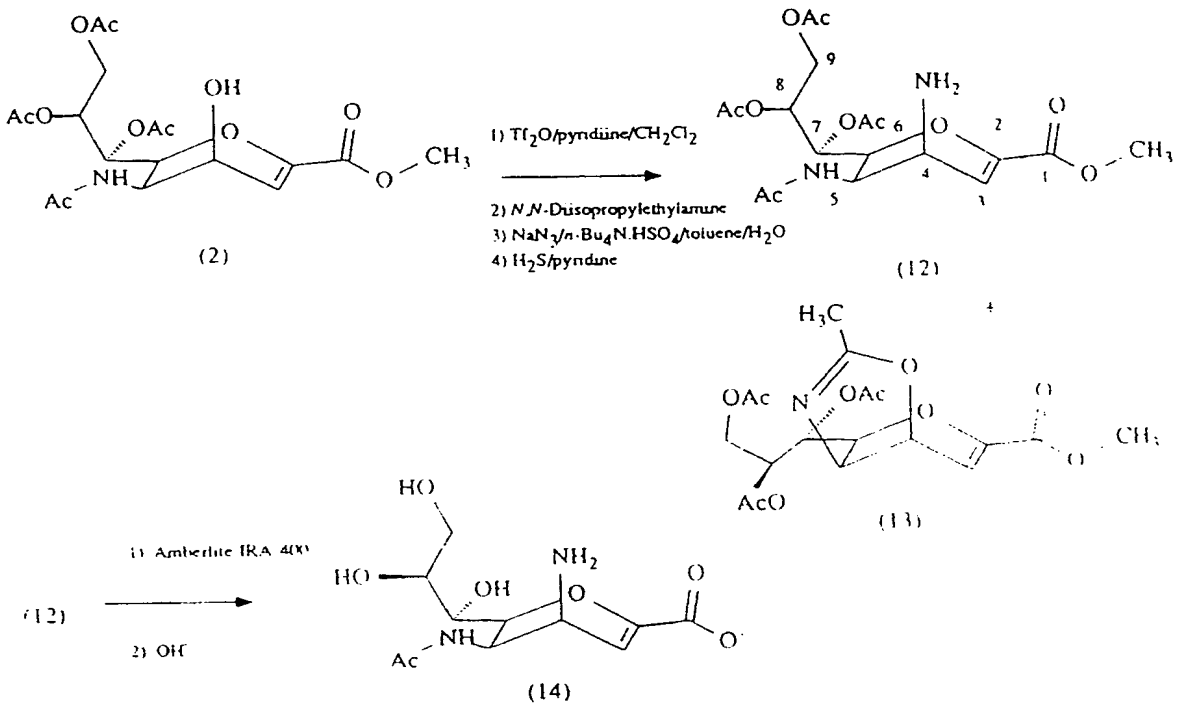
^{1}H-nmr ($CDCl_3$) of compound (10) is shown as follows δ (ppm) 1.96, 2.05, 2.06, 2.11(s, 12H, Acetyl CH_3 x 4), 3.25 (dd, 1H, $J_{10a,10b}$ -14.1Hz, $J_{10a,11}$ 5.8Hz, H_{10a}), 3.37 (dd, 1H, $J_{10b,10a}$ -14.1Hz, $J_{10b,11}$ 5.9Hz, H_{10b}), 3.43 (dd, 1H, $J_{4,3}$ 3.1Hz, $J_{4,5}$ 7.5Hz, H_4), 3.79 (s, 3H, $COOCH_3$), 4.09 (ddd, 1H, $J_{5,4}$ 7.5Hz, $J_{5,NH}$ 9.1Hz, $J_{5,6}$ 8.1Hz, H_5), 4.21 (dd, 1H, $J_{9',8}$ 7.1Hz, $J_{9',9}$ -12.2Hz, $H_{9'}$), 4.30 (dd, 1H, $J_{6,5}$ 8.1Hz, $J_{6,7}$ 4.1Hz, H_6), 4.63 (dd, 1H, $J_{9,8}$ 3.2Hz, $J_{9,9'}$ -12.2Hz, H_9), 5.09 (dd, 1H, $J_{12cis,11}$ 10.2Hz, $J_{12cis,12trans}$ -1.3Hz, H_{12cis}), 5.18 (dd, 1H, $J_{12trans,11}$ 17.1Hz, $J_{12trans,12cis}$ -1.3Hz, $H_{12trans}$), 5.36 (ddd, 1H, $J_{8,7}$ 4.2Hz, $J_{8,9}$ 3.2Hz, $J_{8,9'}$ 7.1Hz, H_8), 5.57 (dd, 1H, $J_{7,6}$ 4.1Hz, $J_{7,8}$ 4.2Hz, H_7), 5.65 (d, 1H, $J_{NH,5}$ 9.1Hz, -CONH-), 5.83 (dddd, 1H, $J_{11,12trans}$ 17.1Hz, $J_{11,12cis}$ 10.2Hz, $J_{11,10a}$ 5.8Hz, $J_{11,10b}$ 5.9Hz, H_{11}), 6.09 (d, 1H, $J_{3,4}$ 3.1Hz, H_3).

Compound (10) (50mg, 0.11mmol) was stirred in anhydrous methanol (5mL) containing sodium methoxide (12mg, 0.225mmol) at room temperature for 2 h, then evaporated to dryness. The residue was redissolved in water (5mL) and allowed to stand at room temperature for 2 h before being neutralized with Dowex 50 x 8 (H^+) resin. The aqueous solution was freeze-dried to afford compound (11) (31mg, 78%).

^{1}H-nmr (D2O) δ (ppm) 2.02 (s, 3H, CH_3CO), 3.42 (dd, 1H, $J_{10a,10b}$ -13.4Hz, $J_{10a,11}$ 6.6Hz, H_{10a}), 3.52 (dd, 1H, $J_{10b,10a}$ -13.4Hz, $J_{10b,11}$ 6.3Hz, J_{10b}), 3.51-4.27 (m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.30 (dd, 1H, $J_{12cis,12trans}$ ~1.5Hz, $J_{12cis,11}$ 10.3Hz, H_{12cis}), 5.34 (dd, 1H, $J_{12trans,12cis}$ ~1.5Hz, $J_{12trans,11}$ 17.7Hz, $H_{12trans}$), 5.72 (d, 1H, $J_{3,4}$ 2.4Hz, H_3), 5.89 (dddd, $J_{11,10a}$ 6.6Hz, $J_{11,10b}$ 6.3Hz, $J_{11,12cis}$ 10.3Hz, $J_{11,12trans}$ 17.7Hz, H_{11}).

Example 6 Sodium 5-Acetamido-4-amino-2,3,4,5-tetra deoxy-D-glycero-D-talo-non-2-enopyranosonate (14).

The overall reaction scheme is as follows:



To a stirred solution of compound (2) (500mg, 1.04mmol) in anhydrous dichloromethane (8mL) containing pyridine (205mg, 2.6mmol) at -30°, was added dropwise a solution of trifluoromethanesulphonic anhydride (Tf2O) (367mg, 1.3mmol) in dichloromethane (2mL) over a period of 20 minutes. The reaction mixture was then stirred at -30° for 5 h, and finally evaporated to dryness under reduced pressure. The resulting residue was stirred in dry DMF containing N,N-diisopropylethylamine (194mg, 1.5mmol) at room temperature for 16 h. The reaction mixture was concentrated under high vacuum to remove DMF. The residue was then stirred in a two-phase mixture of toluene (5mL) and water (5mL) containing tetra-n-butylammonium hydrogen sulphate (950mg, 2.8mmol) and sodium azide (137mg, 2.1mmol). The mixture was stirred at room temperature for 16 h and then evaporated to dryness. The residue was partitioned between

ethyl acetate (50mL) and water (15mL), with the organic layer washed successively with water (5mL x 2), and then evaporated to dryness. The residue was taken up in pyridine (5mL), bubbled with H_2S, and then evaporated to dryness. The residue was subjected to flash-column chromotography (silica gel, the first solvent system was ethyl acetate, the second solvent system was ethyl acetate/iso-propanol/H_2O : 5/2/1). The ethyl acetate eluate contained compound (13) (260mg, 53%). The fractions with a positive ninhydrin reaction, collected from the second solvent system, were combined and evaporated to dryness to afford compound (12) (32mg, 6.5%).

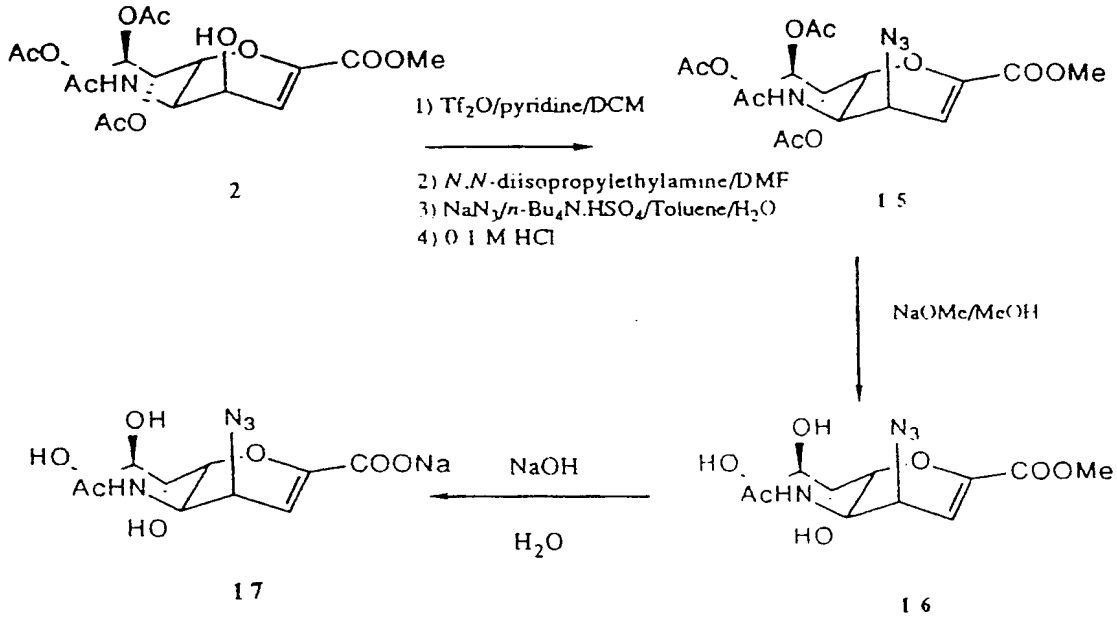
MS (FAB), 431 (M^+ + 1), 414 (M^+ - NH2).
^{1}H-nmr ($CDCl_3$ + CD_3OD) δ (ppm) 1.96, 2.06, 2.08, 2.09 (s, 12H, Acetyl CH_3 x 4), 3.52 (dd, 1H, $J_{4,3}$ 5.5Hz, $J_{4,5}$ 4.5Hz, H_4), 3.80 (s, 3H, $COOCH_3$), 4.16 (dd, 1H, $J_{6,5}$ 10.2Hz, $J_{6,7}$ 2.3Hz, H_6), 4.17 (dd, 1H, $J_{9',9}$-12.4Hz, $J_{9',8}$ 7.3Hz, $H_{9'}$), 4.23 (dd, 1H, $J_{5,6}$ 10.2Hz, $J_{5,4}$ 4.5Hz, H_5), 4.73 (dd, 1H, $J_{9,9'}$-12.4Hz, $J_{9,8}$ 2.7Hz, H_9), 5.34 (ddd, 1H, $J_{8,7}$ 4.7Hz, $J_{8,9}$ 2.7Hz, $J_{8,9'}$ 7.3Hz, H_8), 5.45 (dd, 1H, $J_{7,6}$ 2.3Hz, $J_{7,8}$ 4.7Hz, H_7), 6.12 (d, 1H, $J_{3,4}$ 5.5Hz, H_3).

^{13}C-nmr ($CDCl_3$ + CD_3OD) δ (ppm) 20.7 ($CH_3C(O)O$-), 23.1($CH_3C(O)N$-), 43.8(C_5), 46.2(C_4), 52.4($COOCH_3$), 62.3(C_9), 68.3, 71.8(C_7, C_8), 73.0(C_6), 111.5(C_3), 143.8(C_2), 162.4(C_1), 170.3 & 170.8(CH_3CO x 4).

Compound (12) was stirred in anhydrous methanol (5mL) containing Amberlite IRA-400 (OH-) resin (100mg) at room temperature for 3 h. Following filtration, the filtrate was evaporated to dryness. The residue was dissolved in water (5mL) and adjusted to pH13 with 0.1M NaOH. The aqueous solution was stirred at room temperature for 2 hr and then neutralized with Dowex 50 x 8 (H^+) resin. After filtration, the filtrate was lyophilized to afford compound (14) (16mg, 70%), which was positive in the ninhydrin reaction.

^{1}H-nmr (D_2O) δ (ppm) 2.10 (s, 3H, CH_3CO), 3.67-3.76 (m, 2H, H_4 & $H_{9'}$), 3.92 (dd, 1H, $J_{9,8}$ 2.8Hz, $J_{9,9'}$-11.9Hz, H_9), 3.90-4.02 (m, 2H, H_7 & H_8), 4.37-4.44 (m, 2H, H_5 & H_6), 5.81 (d, 1H, $J_{3,4}$ 5.14Hz, H_3).

Example 7 Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (17).



To a stirring solution of compound (2) (500 mg,1.04 mmol) in anhydrous dichloromethane (8 mL) containing pyridine (205 mg, 2.6 mmol) at -30°C, a solution of trifluoromethane-sulphonic anhydride (Tf_2O) (367 mg, 1.3 mmol) in dichloro-methane (2 mL) was added dropwise over a period of 20 minutes. The reaction mixture was then stirrred at 3°C for 5 h, and finally evaporated to dryness under reduced pressure. The resulting residue was stirred in dry DMF containing N,N-diisopropylethylamine (194 mg, 1.5 mmol) at room temperature for 16 h. The reaction mixture was concentrated under high vacuum to remove DMF. The residue was then stirred in a two-phase mixture of toluene (5 mL) and water (5 mL) containing tetra-n-butylammonium hydrogen sulphate (950 mg, 2.8 mmol) and sodium azide (137 mg, 2.1 mmol). The

mixture was stirred at room temperature for 16 h and then was diluted with 0.2 M HCl (5 mL). The mixture was stirred at room temperature for 48 h. To this reaction mixture were added ethyl acetate (50 mL) and 2 M HCl (1 mL). The organic layer was separated and washed with water (5 mL X 3), then evaporated to dryness. The residue was subjected to flash column-chromatography (silica gel, ethyl acetate/hexane=2/1). The fractions with R_f value of 0.32 (ethylacetate/hexane=2/1 as developing solvent) were combined and evaporated to dryness to afford compound (15). (40 mg, 8.4%). The column was then eluted with ethyl acetate/methanol=10/1 to recover the starting material (2) (280 mg,56%). Compound (15) was isolated as a white foam substance.

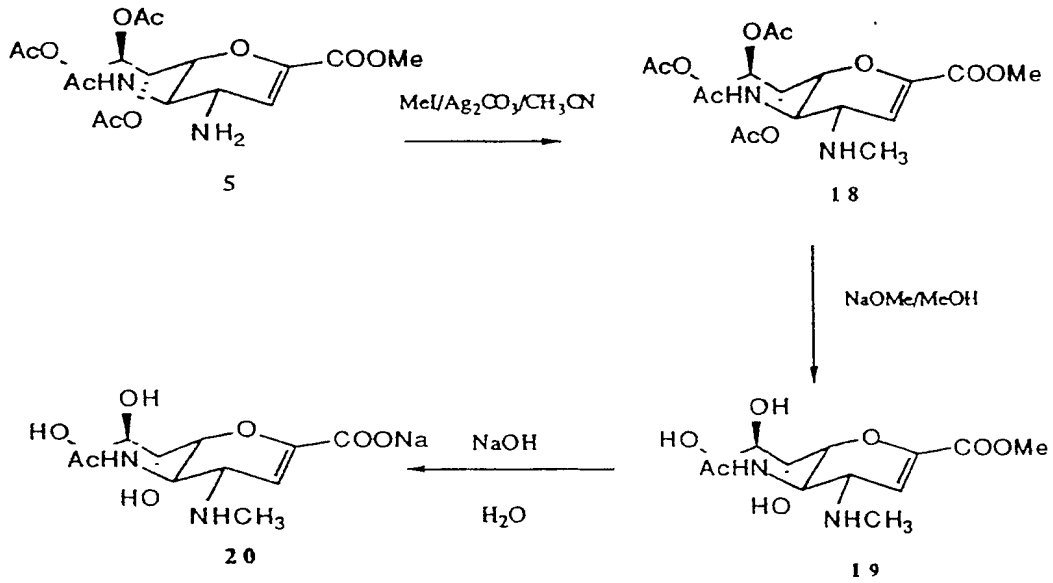
MS (FAB) 457 (M^++1), 414 (M^+-N3),
i.r. ($CHCl_3$) cm^{-1} 2108 ($-N_3$), 1748 (carbonyl)
^{1}H-nmr ($CDCl_3$), δ (ppm)1.97, 2.04, 2.06, 2.07 (s,12H, acetyl CH_3 x 4), 3.82 (s, 3H, $COOCH_3$), 4.12~4.20 (m, 3H,C_6, C_4 & C_9), 4.51 (ddd, 1H, $J_{5,4}$ 4.4Hz, $J_{5,6}$ 10.7Hz, $J_{5,NH}$, 10.1Hz, H_5), 4.69 (dd, 1H, $J_{9,8}$ 2.6Hz, $J_{9,9'}$ 12.4Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 4.9Hz, $J_{8,9}$ 2.6Hz, $J_{8,9'}$ 7.0Hz, H_8), 5.45 (dd, 1H, $J_{7,6}$ 2.1Hz, $J_{7,8}$ 4.9Hz, H_7), 5.68 (d, 1H, $J_{NH,5}$ 10.1Hz,CONH), 6.15 (d, 1H, $J_{3,4}$ 5.7Hz, H_3)

^{13}C-nmr ($CDCl_3$) δ (ppm)
20.7, 20.8, (CH_3CO-O x 3), 23.1 (O CH_3 CO-NH), 44.8 (C_5), 52.6 ($COOCH_3$), 54.8 (C_4), 62.1 (C_9), 67.6, 71.3 (C_7, C_8), 73.5 (C_6), 104.5 (C_3), 146.3 (C_2), 161.5 (C_1), 169.9, 170.2, 170.5 (acetyl, -C=O X 4)

Compound (15) (40 mg, 0.088 mmol) was dissolved in anhydrous methanol (4 mL) containing sodium methoxide (6.4 mg, 0.12 mmol). The mixture was stirred at room temperature for 2 h and concentrated to dryness in vacuo to afford compound (16), which was then dissolved in water (3 mL), stirred at room temperature for 2 h, adjusted to pH 6~7 with Dowex 50 X 8 (H^+) resin, and then lyophilised to give the title compound (17) as a yellowish powder (25 mg, 83%).

i.r. (KBr) cm^{-1} 3400 (br, -OH), 2108 ($-N_3$), 1714 (carbonyl)
^1H-nmr (D_2O) δ (ppm)
1.97 (s, 3H, acetyl), 3.5~4.4 (m, 7H, H_4, H_5, H_6,H_7, H_8, H_9, & H_9'), 6.07 (d, $J_{3,4}$ 5.6Hz, H_3)

Example 8 Sodium 5-acetamido-4-N-methylamino-2,3,4.5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (20)



To a solution of methyl iodide (15 mg, 0.10 mmol) and compound (5) (48 mg, 0.11 mmol) in acetonitrile (6 mL) was added silver carbonate (42 mg, 0.15 mmol). The mixture was stirred whilst protected from light, at room temperature for 16 h. The resulting suspension was filtered off, and the filtrate was evaporated to dryness. The residue was subjected to chromatography (silica gel, ethyl acetate/isopropanol/water=5/2/1). Fractions with R_f value of 0.36 were combined and concentrated in vacuum to dryness to afford compound (18) (25 mg, 51%).

MS (FAB) 445 (M^++1), 414 (M^+ - $NHCH_3$)

^1H - nmr ($CDCl_3$) δ (ppm)

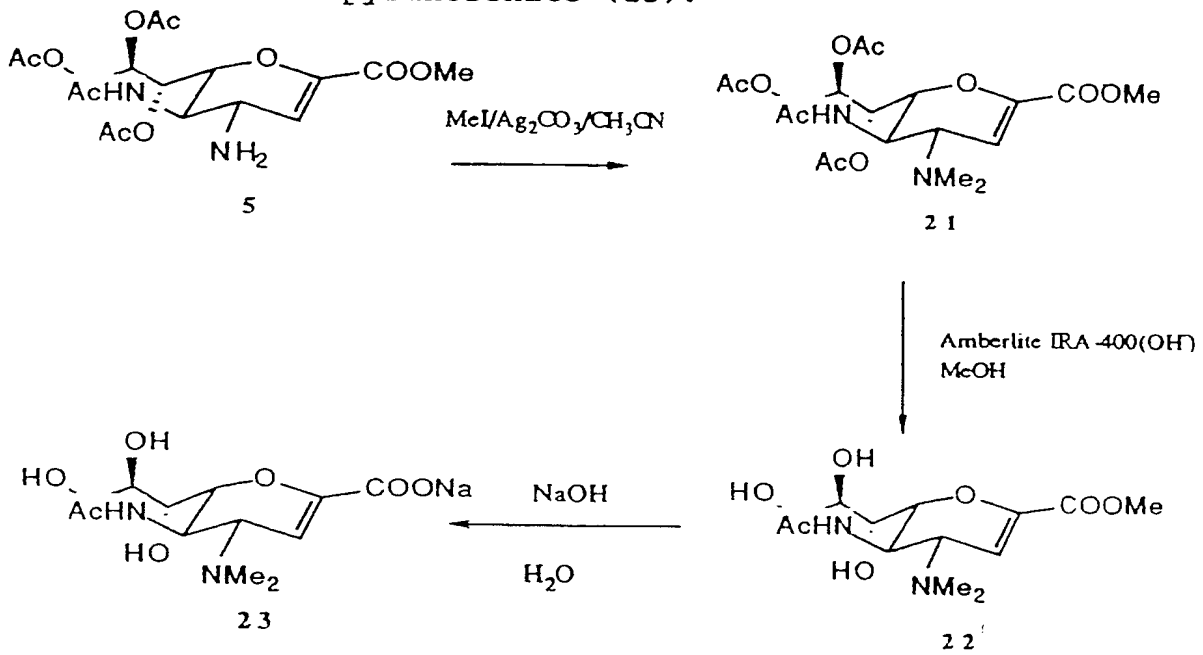
1.95, 2.05, 2.06, 2.12 (s, 12H, acetyl CH_3 X 4), 2.45 (s, 3H, N-CH_3), 3.72 (dd, 1H, $J_{4,3}$ 2.3Hz, $J_{4,5}$ 9.2Hz, H_4), 3.89 (s, 3H, $COOCH_3$), 4.16 (dd, 1H, $J_{9',8}$ 7.2Hz, J9',9 12.3Hz, $H_{9'}$), 4.26 (ddd, 1H, $J_{5,4}$ 9.2Hz, $J_{5,NH}$ 9.1Hz, $J_{5,6}$ 9.0Hz, H_5), 4.36 (dd, 1H, $J_{6,5}$ 9.0Hz, $J_{6,7}$ 2.7Hz, H_6), 4.64 (dd, 1H, $J_{9,8}$ 2.9Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.34 (m, 1H, $J_{8,7}$ 4.8Hz, $J_{8,9}$ 2.9Hz, $J_{8,9'}$ 7.2Hz, H_8), 5.51 (dd, 1H, $J_{7,6}$ 2.7Hz, $J_{7,8}$ 4.8Hz), 6.05 (d, 1H, $J_{3,4}$ 2.3Hz, H_3)

Compound (18) (25 mg, 0.056 mmol) was stirred in anhydrous methanol (5 mL) containing sodium methoxide (5.4 mg, 0.1 mmol) at room temperature for 2 h, then evaporated to dryness to give compound (19), which was redissolved in water (5 mL) and allowed to stand at room temperature for 2 h before being neutralized with Dowex 50 x 8 (H^+) resin. The filtrate was lyophilised to afford compound (20) (15 mg, 82%).

^1H-nmr (D_2O) δ (ppm)

1.94 (s, 3H, CH_3CO), 2.43 (s, 3H, N-CH_3), 3.5~4.3 (m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.65 (d, 1H, $J_{3,4}$ 2Hz, H_3)

Example 9 Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (23).



5 23 22

To a solution of methyl iodide (65 mg, 0.46 mmol) and compound (5) (100 mg, 0.23 mmol) in acetonitrile (15 mL) was added silver carbonate (127 mg, 0.46 mmol). The mixture was stirred and protected from light at room temperature for 16 h. The resulting suspension was filtered off and the filtrate was evaporated to dryness. The residue was subjected twice to flash-column chromatography (silica gel, ethyl acetate/ isopropanol/water=5/2/1) to afford compound (21) (30 mg, 28%) as a colourless foam.

MS (FAB) 459 (M^++1) 414 (M^+-$N(CH_3)_2$)

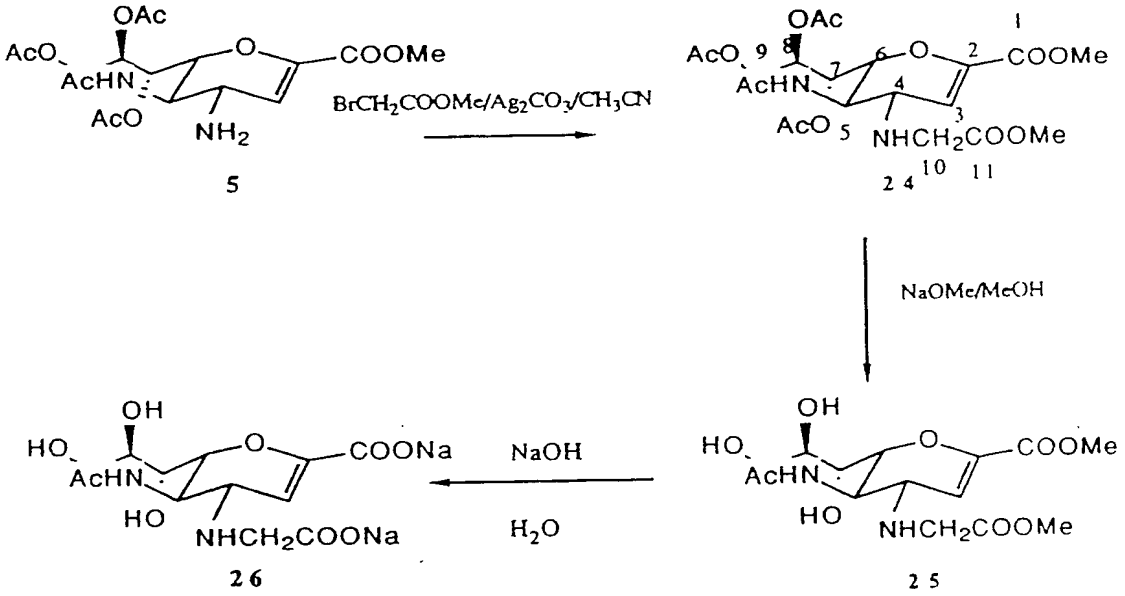
^{1}H-nmr ($CDCl_3$) δ (ppm)

1.98, 2.05, 2.06, 2.12 (s, 12H, acetyl, CH_3 X 4), 2.33 (br s, 6H, $N(CH_3)_2$), 3.42 (dd, 1H, $J_{4,3}$ 2.8 Hz, $J_{4,5}$ 8.6Hz, H_4), 3.79 (s, 3H, $COOCH_3$), 4.17 (dd, 1H, $J_{9'8}$ 7.4Hz, $J_{9',9}$ 12.3Hz, H_9'), 4.18 (ddd, 1H, $J_{5,4}$ 8.5Hz, $J_{5,NH}$ 8.9Hz, $J_{5,6}$ 9.0Hz, H_5), 4.31 (dd, 1H, $J_{6,5}$ 9.0Hz, $J_{6,7}$ 2.9Hz, H_6), 4.68 (dd, 1H, $J_{9,8}$ 3.0Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.31 (m, 1H, $J_{8,7}$ 4.4Hz, $J_{8,9}$ 3.0Hz, $J_{8,9'}$ 7.4Hz, H_8), 5.51 (dd, 1H, $J_{7,6}$ 2.9Hz, $J_{7,8}$ 4.4Hz, H_7), 5.79 (d, 1H, $J_{NH,5}$ 8.9Hz, CONH), 6.09 (d, 1H, $J_{3,4}$ 2.8Hz, H_3)

Compound (21) (30 mg, 0.066 mmol) was stirred in anhydrous methanol (4 mL) containing dry Amberlite IRA 400 (OH^-) resin (90 mg) at room temperature for 3 h, then the resin filtered off. The filtrate and washings were combined and evaporated to dryness to afford compound (22) (20 mg), which was stirred in water (5 mL) at pH 12 at room temperature for 2 h, then was adjusted to pH 7.5 with Dowex 50 X 8 (H^+) before filtration. The filtrate was lyophilised to afford compound (23) (15 mg, 66%) as a white powder.

^{1}H-nmr (D_2O) δ (ppm)
1.97 (s, 3H, acetyl), 2.33 (s, 6H, $N(CH_3)_2$), 3.50~4.26 (m, 7H, H_4, H_5, H_6, H_7, H_8, H_9 & $H_{9'}$), 5.71 (d, $J_{3,4}$ 1.8Hz, H_3)

Example 10 Disodium 5-acetamido-4-N-oxycarbonylmethyl-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (26).



To a solution of methyl α-bromoacetate (36 mg, 0.23 mmol) and compound (5) (100 mg, 0.23 mmol) in acetonitrile (12 mL) was added silver carbonate (64 mg, 0.23 mmol). The mixture was stirred at room temperature for 16 h whilst shielded from light, then filtered. The filtrate was

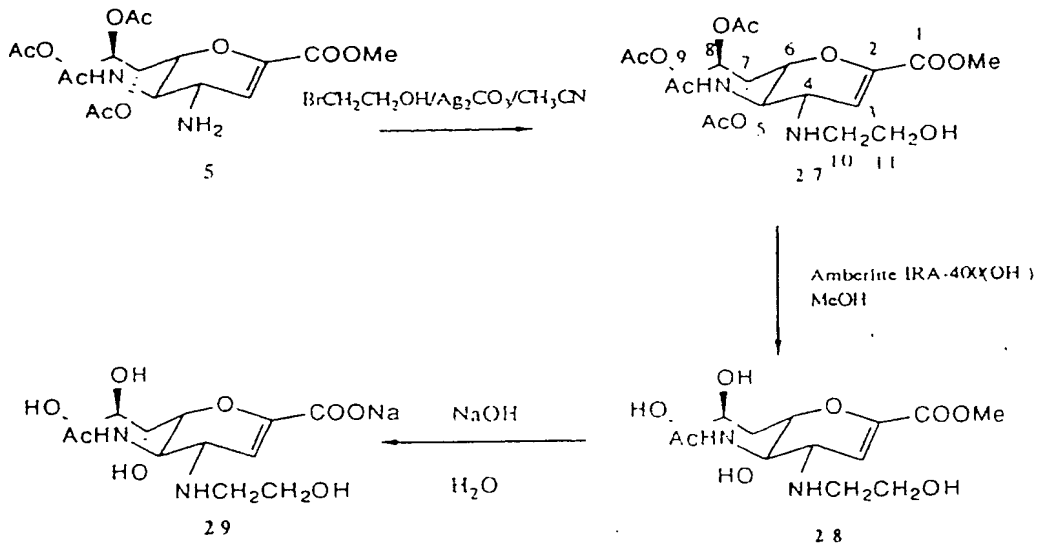
evaporated to dryness. The residue was chromatographed on silica-gel column (ethyl acetate/isopropanol/water=5/2/1). Fractions with R_f value of 0.60 were collected and evaporated to dryness to afford compound (24) (80 mg, 68.5%).

^{1}H-nmr ($CDCl_3$) δ (ppm)
1.97, 2.044, 2.047, 2.11 (s, 12H, acetyl CH_3 x 4), 3.49 (AB, 2H, J_{AB} 17.6Hz, H_{10} x 2), 3.50 (dd, 1H, $J_{4,3}$ 2.9Hz, $J_{4,5}$ 8.4Hz, H_4), 3.71 (s, 3H,C_{11}OOMe), 3.79 (s, 3H,C_1OOMe), 4.09 (ddd, 1H, $J_{5,4}$ 8.4 Hz, $J_{5,NH}$ 8.8Hz, $J_{5,6}$ 8.1Hz,H_5), 4.17 (dd, 1H, $J_{9',8}$ 7.4Hz, $J_{9',9}$ 12.3Hz, $H_{9'}$), 4.32 (dd, 1H, $J_{6,5}$ 8.1Hz, $J_{6,7}$ 4.1Hz, H_6), 4.63 (dd, 1H, $J_{9,8}$ 3.1Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.37 (m, 1H, $J_{8,7}$ 4.1Hz, $J_{8,9}$ 3.1Hz, $J_{8,9'}$ 7.4Hz, H_8), 5.56 (t, 1H, $J_{7,6}$ 4.1Hz, $J_{7,8}$ 4.1Hz, H_7), 6.03 (d, 1H, $J_{NH,5}$ 8.8Hz, CONH), 6.04 (d, 1H, $J_{3,4}$ 2.9Hz, H_3)

Compound (24) (80 mg, 0.159 mmol) was stirred in anhydrous methanol (20 mL) containing sodium methoxide (18 mg, 0.32 mmol) at room temperature for 2 h, then evaporated to dryness to give compound (26), which was redissolved in water (15 mL). The solution was allowed to stand at room temperature for 2 h before being adjusted to pH 7 by Dowex 50 x 8 (H^+) resin. The filtrate was freeze-dried to afford compound (25) as a white powder (59 mg, 94.6%).

^{1}H-nmr (D_2O) δ (ppm)
2.04 (s, 3H, acetyl), 3.58 (AB, 2H, J_{AB} 17.6 Hz, H_{10} x 2), 3.50~4.40 (M, 7H, H_4, H_5, H_6, H7, H_8, H_9 & $H_{9'}$), 5.68 (d, 1H, $J_{3,4}$ 2.1Hz, H_3)

Example 11 Sodium 5-acetamido-4-N-2'-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (29)



5 27 28 29

To a solution of bromoethanol (158 mg, 1.26 mmol) and compound (5) (84 mg, 0.195 mmol) in acetonitrile (10 mL) was added silver carbonate (100 mg, 0.36 mmol). The mixture was protected from light and stirred at room temperature for 7 days. Then it was filtered off, the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (ethyl acetate/isopropanol/water=5/2/1). Fractions with R_f value of 0.4 were combined and evaporated to dryness to afford compound (27) (40 mL, 40%).

MS (FAB) 475 (M^++1), 414 (M^+-$NHCH_2CH_2OH$)

^{1}H-nmr ($CDCl_3$) δ (ppm)

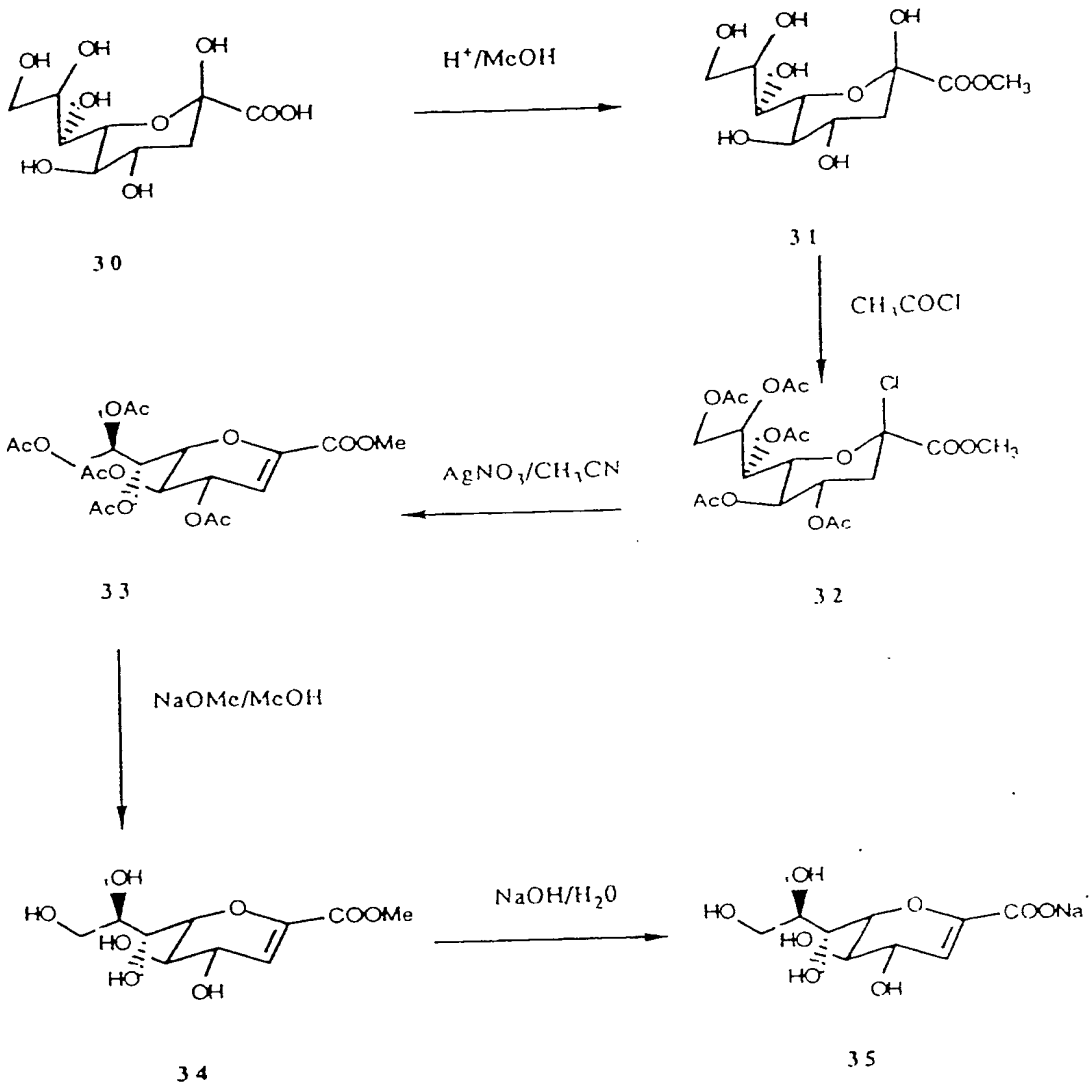
1.96, 2.05, 2.10 (s, 12H, acetyl CH_3 x 4), 2.29 (br. s, 2H, NH &OH), 2.76 (ABm, 2H, H_{10} X 2), 3.47 (dd, 1H, $J_{4,3}$ 2.9Hz, $J_{4,5}$ 7.5Hz, H_4), 3.62 (t, 2H, $J_{11,10}$ 4.9Hz, H_{11} x 2), 3.79 (s, 3H, $COOCH_3$), 4.15 (ddd, 1H, $J_{5,4}$ 7.5Hz, $J_{5,6}$ 8.4Hz, $J_{5,NH}$ 8.3Hz, H_5), 4.19 (dd, 1H, $J_{9',8}$ 7.5Hz, $J_{9',9}$ 12.3Hz $H_{9'}$), 4.29 (dd, 1H, $J_{6,5}$ 8.4Hz, $J_{6,7}$ 3.8Hz, H_6), 4.65 (dd, 1H, $J_{9,8}$ 2.9Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.36 (m, 1H, $J_{8,7}$ 4Hz, $J_{8,9}$ 2.9Hz, $J_{8,9'}$ 7.5Hz, H_8), 5.55 (dd, 1H, $J_{7,6}$ 3.8Hz, $J_{7,8}$ 4Hz, H_7), 6.08 (d, 1H, $J_{3,4}$ 2.9Hz, H_3), 6.09 (d, 1H, $J_{NH,5}$ 8.3Hz, CONH)

^{13}C-nmr (CDCl3) δ (ppm)
20.6, 20.8, (CH_3-CO-O- x 3), 23.10 (CH_3-Co-NH), 46.5 (C_5), 47.2 (C_{10}), 52.3 (CH_3COOCH_3), 55.6 (C_4), 61.1 (C_{11}), 62.1 (C_9), 68.1, 71.1 (C_7,C_8), 76.7 (C_6), 111.6 (C_3), 143.7 (C_2), 162.1 (C_1), 170.1, 170.3, 170.6, 171.0 (acetyl carbonyl x 4)

Compound (27) (40 mg, 0.084 mmol) was stirred in anhydrous methanol (10 mL) containing dry Amberlite IRA-400 (OH^-) (120 mg) at room temperature for 4 h, then filtered. The filtrate and washings were combined and evaporated to dryness to give compound (28), which was redissolved in water (10 mL) and adjusted to pH 13 by adding NaOH. The aqueous solution was left at room temperature for 3 h before being adjusted to pH 6™7 with Dowex 50 x 8 (H^+) resin. The solution after filtration was lyophilised to afford compound (29) as a white powder (20 mg 66%).

^{1}H-nmr (D_2O) δ (ppm)
1.99 (s, 3H, acetyl), 2.91 (AB, 2H, H_{10} x 2), 3.53 ~ 4.25 (m, 9H, H_4, H_5, H_6, H_7, H_8, H_9, $H_{9'}$, H_{11} X 2), 5.65 (d, $J_{3,4}$ 2.24 Hz, H_3)

Example 12 Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate (35)



Compound (30) (332 mg, 1.24 mmol) was stirred in anhydrous methanol (40 mL) containing Dowex 50 x 8 (H^+) resin (50 mg) at room temperature for 16 h before filtration. The filtrate was evaporated to dryness to give compound (31) (320 mg, 1.13 mmol, 91.5%), which was stirred in acetyl chloride (5 mL) at room temperature for 3 days then evaporated to dryness to afford Compound (32) (539 mg, 1.057 mmol, 93.6%). The residue was dissolved in acetonitrile (20 mL) containing silver nitrate (500 mg, 2.94 mmol) and potassium carbonate

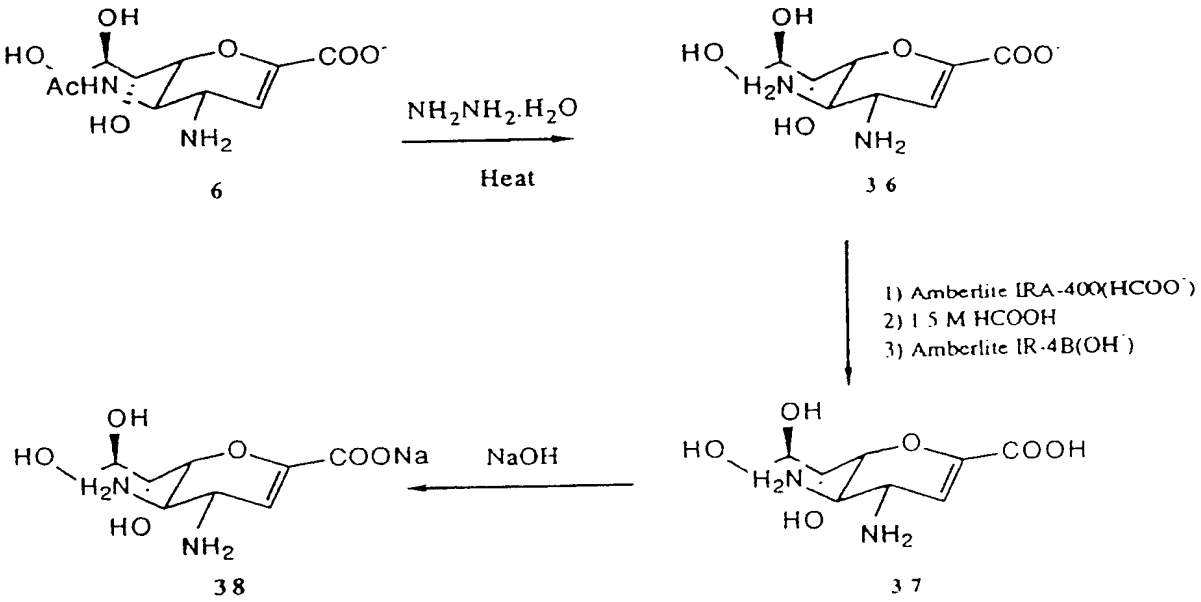
(90 mg, 0.65 mmol) protected from light and stirred at room temperature for 16 h, then filtered. The filtrate was evaporated to small volume and partitioned between ethyl acetate (75 mL) and water (15 mL). The organic layer was washed with water (10 mL x 3) and evaporated to dryness. The residue was chromatographed on silica gel column (ethyl acetate/hexane=2/1) to afford pure compound (33) (200 mg, 0.423 mmol, 40%).

^{1}H-nmr ($CDCl_3$) δ (ppm)
2.062, 2.070, 2.073, 2.094, 2.096 (s, 15H, acetyl CH_3 x 5), 3.80 (s, 3H, $COOCH_3$), 4.19 (dd, 1H, $J_{9,8}$ 5.9Hz, $J_{9',9}$ 12.3Hz, $H_{9'}$), 4.33 (dd, 1H, $J_{6,5}$ 9.4Hz, $J_{6,7}$ 3.0Hz, H_6), 4.57 (dd, 1H, $J_{9,8}$ 1.9Hz, $J_{9,9'}$ 12.3Hz, H_9), 5.20 (dd, 1H, $J_{5,4}$ 7.0Hz, $J_{5,6}$ 9.4Hz, H_5), 5.38 (m, 1H, $J_{8,7}$ 5.1Hz, $J_{8,9}$ 1.9Hz, $J_{8,9'}$ 5.9Hz H_8), 5.49 (dd, 1H, $J_{7,6}$ 3.0Hz, $J_{7,8}$ 5.1Hz, H_7), 5.57 (dd, 1H, $J_{4,3}$ 3.1Hz, $J_{4,5}$ 7.0Hz, H_4), 5.97 (d, 1H, $J_{3,4}$ 3.1Hz, H_3)

Compound (33) (100 mg, 0.211 mmol) was stirred in anhydrous methanol (10 mL) containing sodium methoxide (24 mg, 0.423 mmol) at room temperature for 3 h, then evaporated to dryness to afford compound (34) (50 mg, 90%), which was redissolved in water (5 mL) and left at room temperature for 3 h before adjusted to pH 7 with Dowex 50 x 8 (H^+) resin. The solution was freeze-dried to give compound (35) (47 mg, 91%).

^{1}H-nmr (D_2O) δ (ppm)
3.69 (dd, 1H, $J_{9',8}$ 5.6Hz, $J_{9',9}$ 12.0Hz, $H_{9'}$), 3.76 (dd,1H, $J_{5,4}$ 7.8Hz, $J_{5,6}$ 10.5Hz H_5), 3.87 ~ 3.99 (m, 3H, H_7, H_8, H_9), 4.13 (d, 1H, $J_{6,5}$ 10.5Hz, H_6), 4.40 (dd, 1H, $J_{4,3}$ 2.3Hz, $J_{4,5}$ 7.8Hz, H_4), 5.67 (d, 1H, $J_{3,4}$ 2.3Hz H_3)

Example 13 Sodium 4,5-Diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (38)



A solution of compound (6) (125 mg, 0.40 mmol) in hydrazine hydrate (5 mL) under argon was heated at 85°C for 3 days, and the resulting mixture was vacuum evaporated to dryness. The residue was dissolved in water (15 mL) and passed through a column of Amberlite IRA-400 ($HCOO^-$), then eluted with 1.5 M HCOOH. The eluate (200 mL) was evaporated to dryness. The residue was chromatographed on silica gel deactivated with 10% water (developing solvent: isopropanol/water = 4/1). The fractions with R_f value of 0.1 were combined and evaporated to dryness, then freeze-dried. The residue, compound (36), was dissolved in water (10 mL), passed through a small column of Amberlite IR-4B (OH^-)(10 mL). The effluent was evaporated to dryness to give compound (37), MS (FAB) of which was 249 (M^++1). Compound (37) was dissolved in water and adjusted to pH 7.5 with 0.1 M NaOH, then freeze-dried to afford compound (38) (20 mg, 20%) as a white powder.

^{1}H-nmr (D_2O) δ (ppm)
3.01 (dd, 1H, $J_{5,4}$ 9.7Hz, $J_{5,6}$ 10.2Hz, H_5), 3,58 (m, 2H, H_9, & H_7), 3.80™3.89 (m, 3H, H_4, H_8, & H_9), 4.06 (d, 1H, $J_{6,5}$ 10.2Hz, H_6), 5.54 (d, 1H, $J_{3,4}$ 2.4Hz, H_3)

Example 14 Methyl 5-acetamido-2,3,5-trideoxy-9-(p-toluenesulphonyl)-D-glycero-D-galacto-non-2-enopyranosonate (39).

A solution made up of methyl 5-acetamido-2,3,5-trideoxy-D-glycero-D-galacto-non-2-enopyranosonate (1000 mg., 3.16 mmol) in dry pyridine (85 mL) was cooled in an ice-bath. p-Toluenesulphonyl chloride (660 mg., 3.46 mmol) was added and the pale yellow homogeneous solution left to stir overnight at 4°C.

Further p-toluenesulphonyl chloride (220 mg., 1.15 mmol) was added and the solution left to stir for an additional 4h at room temperature.

Workup was first by addition of water (1 mL) followed by rotary evaporation to afford a viscous yellow oil which was flash chromatographed (SiO_2, EtOAc/i-PrOH/H_2O, 6/2/1,v/v/v) to give as the major product 1.19 g. (80% yield) of compound (39).

i.r (KBr) : ν_{max} (cm^{-1}) 2964 (OH), 1730 (CO_2CH_3), 1656 (NHAc),
1358, 1174 (SO_2), 810, 662, 550 (Ar)
MS (FAB); 460 ($M+H^+$)
^{1}H nmr (300 MHz, CD_3OD/TMS); δ (ppm) = 2.03 (s, 3H, NHAc), 2.45 (s, 3H, $ArCH_3$), 3.49 (d, 1H, $J_{6,7}$ 1.70, H_6), 3.76 (s, 3H, CO_2CH_3),
3.91 (dd, 1H, $J_{5,6}$ 10.80, H_5), 3.98-4.13 (m, 3H, H_8, H_9 and H_9'),
4.28 (dd, 1H, $J_{7,8}$ 9.55, H_7), 4.39 (dd, 1H, $J_{4,5}$ 8.64, H_4), 5.92
(d, 1H, $J_{3,4}$ 2.49, H_3), 7.74 (d, 2H, ArH), 7.79 (d, 2H, ArH)

Example 15 Methyl 5-acetamido-9-azido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (40)

Methyl 5-acetamido-2,3,5-trideoxy-9-(p-toluenesulphonyl)-D-glycero-D-galacto-non-2-enopyranosonate (39) (600 mg., 1.27 mmol) and lithium azide (186 mg., 3.80 mmol) were dissolved in dry DMF (20 mL) and the yellow homogenous solution heated to 80°C. After 2 h, further lithium azide (186 mg., 3.80 mmol) was added and the solution left at 80°C overnight. The solvent was removed by rotary evaporation and the remaining dark brown oil dissolved in pyridine (2 mL) and flash chromatographed (SiO_2, 5/2/1 EtOAc/i-PrOH/H_2O). The major product was compound (40) (370 mg., 88% yield) obtained as a white foam.

i.r.(KBr):ν_{max} (cm^{-1}) 3428 (s,OH), 2104 (s, N_3), 1730 (s, CO_2CH_3), 1656 (s, NHAc)
MS (FAB) : 331 ($M+H^+$)
^1H nmr (300 MHz, D_2O): δ (ppm) = 1.94 (s, 3H, NHAc), 3.37 (dd, 1H, $H_{9'}$),
3.48 - 3.57 (m, 2H, $J_{8,9}$, 5.77, H_8 and $J_{9,9'}$ 13.16, H_9), 3.66 (s, 3H, CO_2CH_3), 3.91 - 3.98 (m, 2H, H_5 and H_6), 4.15 (d, 1H, $J_{7,8}$ 10.86, H_7), 4.38 (dd, 1H, $J_{4,5}$ 8.88, H_4), 5.91 (d, 1H, $J_{3,4}$ 2.44, H_3)

Example 16 Methyl 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (41).

Thiolacetic acid (130 mL, 1.82 mmol) was added to methyl 5-acetamido-9-azido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (70 mg.,0.21 mmol) to give a pale yellow solution that was left to stir overnight at room temperature.

Excess thiolacetic acid was then evaporated off under low pressure and the remaining solid repeatedly treated with water followed by evaporation (3x3 mL). The remaining solid was dissolved in methanol (4 mL), filtered and the

filtrate applied to a preparative tlc plate (SiO_2, 20 cm. x 20 cm. x 2 mm. eluted with 5/2/1 EtOAc/i-PrOH/H_2O). The band with R_f=0.47 was worked up to give 51 mg. (70% yield) of compound (41) as a white powder.

i.r. (KBr) : ν_{max} (cm^{-1}) 3400 (s, OH), 1728 (s,CO_2CH_3), 1656 (s, NHAc)

MS (FAB) : 347 ($M+H^+$)

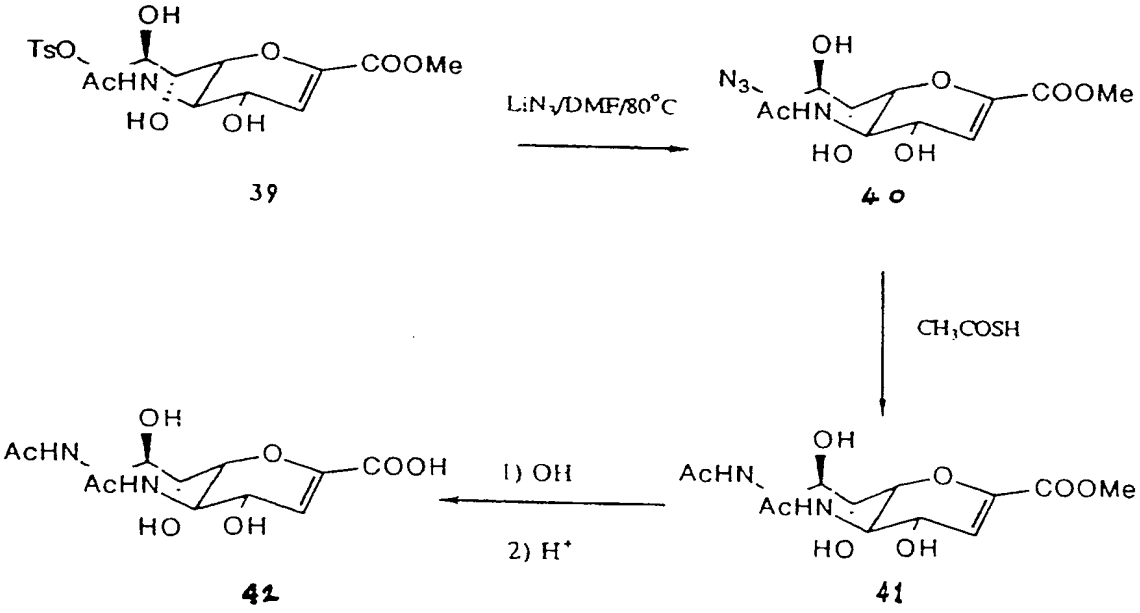
^1H nmr (300 MHz, D_2O) : δ (ppm) = 1.96 (s, 3H, NHAc), 2.00 (s, 3H, NHAc),

3.23 (dd, 1H, H_9'), 3.48 (d, 1H, H_6), 3.56 (dd, 1H, $J_{9,9'}$ 14.17, H_9),

3.75 (s, 3H, CO_2CH_3), 3,89 (m, 1H, $J_{8,9}$ 2.90, $J_{8,9'}$ 7.40, H_8), 4.02 (dd, 1H, $J_{5,6}$ 9.10, H_5), 4.22 (d, 1H, $J_{7,8}$ 10.85, H_7), 4.45 (dd, 1H, $J_{4,5}$ 8.94, H_4), 5.61 (d, 1H, $J_{3,4}$ 2.47, H_3);

Example 17 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (42).

The preparation of compound (42) from compound (39) is summarized below:



A solution of methyl 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (41) (46 mg., 0.13 mmol) dissolved in 0.1M aq. sodium hydroxide (5 mL) was stirred at room temperature for 2.5 h. The solution was then adjusted to pH 5 with Dowex 50W-X8 (H^+), the resin filtered off and the filtrate lyophilized to give 40 mg. (91% yield) of compound (42) as a white powder.

i.r. (KBr) : ν_{max} (cm^{-1}) 3376 (s, OH), 1652 (s, NHAc)
MS (FAB) : 333 ($M+H^+$)
^1H nmr (300 MHz, D_2O) : δ (ppm) = 1.89 (s, 3H, NHAc), 1.93 (s, 3H, NHAc),
3.15 (dd, 1H, $H_{9'}$), 3.40 (d, 1H, H_6), 3.48 (dd, 1H, $J_{9,9'}$ 14.18, H_9),
3.82 (m, 1H, $J_{8,9}$ 3.01, $J_{8,9'}$ 7.43, H_8), 3.94 (dd, 1H, $J_{5,6}$ 10.42, H_5), 4.13 (d, 1H, $J_{7,8}$ 10.91, H_7), 4.36 (dd, 1H, $J_{4,5}$ 8.80, H_4), 5.81 (d, 1H, $J_{3,4}$ 2.41, H_3)

Example 18 Methyl 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (43)

A solution of methyl 5-acetamido-2,3,5-trideoxy-9-(p-toluenesulphonyl)-D-glycero-D-galacto-non-2-enopyranosonate (39) (80 mg., 0.17 mmol), tert-butylammonium cyanide (2 mg) and sodium cyanide (12 mg., 0.25 mmol) in dry DMSO (1.25 mL) was stirred at room temperature for 5 days.

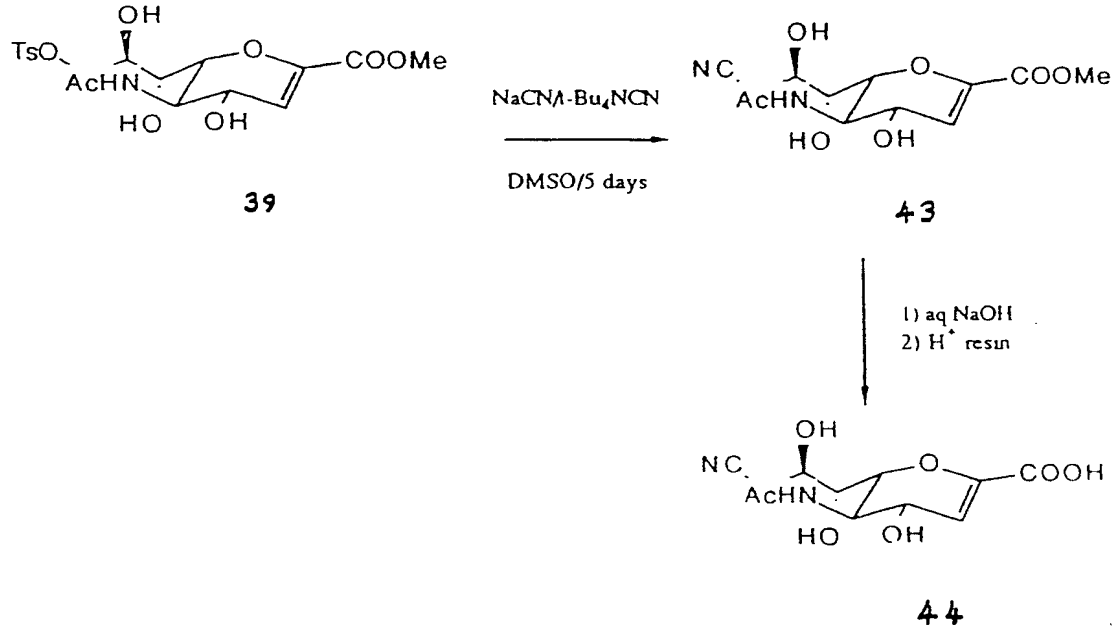
Workup by preparative thin layer chromatography (SiO_2, 20 cm. x 20 cm. x 2 mm. eluted with EtOAc/ i-PrOH/H_2O, 5/2/1) gave as the major component 30 mg. (61% yield) of compound (43) as a cream coloured powder.

(R_f=0.74).
i.r.(KBR) : ν_{max} (cm^{-1}) 3440 (s, OH), 2256 (w, CN), 1726 (s, CO_2CH_3),
1638 (s, NHAc)
MS (FAB) : 315 ($M+H^+$)
^1H nmr (300mHz, D_2O) : δ (ppm) = 1.92 (s, 3H, NHAc), 2.75

(dd, 1H, $H_{9'}$),
2.93 (dd, 1H, $J_{9,9'}$17.22, H_9), 3.55 (dd, 1H, $J_{6,7}$ 1.17, H_6), 3.67 (s, 3H, CO_2CH_3), 4.02 (dd, 1H, $J_{5,6}$ 9.05, H_5), 4.13-4.19 (m, 1H, $J_{8,9}$ 3.91, $J_{8,9'}$6.56, H_8), 4.16 (dd, 1H, $J_{7,8}$ 10.90, H_7), 4.37 (dd, 1H,$J_{4,5}$ 8.95, H_4), 5.90 (d, 1H, $J_{3,4}$ 2.42, H_3)

Example 19 5-Acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (44).

The methodology used to prepare 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (44) is summarised below:



Methyl 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (43) (80 mg., 0.25 mmol) was dissolved in 0.1M aq. sodium hydroxide (10 mL) and the resultant solution stirred at room temperature for 3 h.

The pH was then adjusted to 4 with Dowex 50W-X8(H^+), the resin filtered off and the filtrate lyophilized to give 75 mg (98% yield) of compound (43) as a fluffy white powder.

i.r. (KBr) : ν_{max} (cm^{-1}) 3370 (s,OH), 2254 (w, CN), 1656 (s, NHAc)

MS (FAB) : 301 ($M+H^+$)

^1H nmr (300MHz, D_2O) : δ (ppm) = 1.98 (s, 3H, NHAc), 2.70 (dd, 1H, $H_{9'}$), 2.88 (dd, 1H, $J_{9,9'}$ 17.27, H_9), 3.48 (d, 1H, H_6), 3.97 (dd, 1H, $J_{5,6}$ 9.84, H_5), 4.09-4.24 (m, 2H, H_7 and H_8, $J_{8,9}$ 3.90, $J_{8,9'}$ 6.53), 4.41 (dd, 1H, $J_{4,5}$ 8.87, H_4), 5.80 (d, 1H, $J_{3,4}$ 2.42, H_3)

Example 20 Inhibition of Influenza Virus Neuraminidase

An in vitro bioassay of the above-described compounds against N2 influenza virus neuraminidase was conducted, following Warner and O'Brien, Biochemistry, 1979 18 2783-2787. For comparison, with the same assay the K_i for 2-deoxy-N-acety-α-D-neuraminic acid was determined to be 3 x 10^{-4} M.

Values for K_i were measured via a spectrofluorometric technique which uses the fluorogenic substrate 4-methylumbelliferyl N-acetylneuraminic acid (MUN), as described by Meyers et al., Anal. Biochem. 1980 101 166-174. For both enzymes, the assay mixture contained test compound at several concentrations between 0 and 2 mM, and approximately 1 mU enzyme in buffer (32.5 mM MES, 4 mM $CaCl_2$, pH 6.5 for N2; 32.5 mM acetate, 4 mM $CaCl_2$, pH 5.5 for V. cholerae neuraminidase).

The reaction was started by the addition of MUN to final concentrations of 75 or 40 μM. After 5 minutes at 37°C, 2.4 ml 0.1 M glycine-NaOH, pH 10.2 was added to 0.1 ml reaction mixture to terminate the reaction. Fluorescence was read at excitation 365 nm, emission 450 nm, and appropriate MUN blanks (containing no enzyme) were subtracted from readings. The K_i was estimated by Dixon plots (1/fluorescence versus compound concentration). Results are summarized in Table 1, and unless otherwise stated, refer to inhibition of N2 neuraminidase.

Table 1

Inhibition of Influenza Virus Neuraminidase In Vitro

Compound	Ki (M)
2-deoxy-N-acetyl-α-D-neuraminic acid	3×10^{-4}
sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4)	2×10^{-6}
sodium 5-acetamido-4-amino-2,3,4,5-tetradexoy-D-glycero-D-galacto-non-2-enopyranosonate (6)	6×10^{-8}
	1.9×10^{-7} (N9 neuraminidase, pH 6.5)
	1×10^{-8} (N2 virus, pH 7.5)
ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7)	1.7×10^{-8}
	$> 5 \times 10^{-8}$ (N9 neuraminidase)

	4.5×10^{-4} (V. cholerae neuraminidase; pH 5.8)
	$> 10^{-2}$ (sheep neuraminidase; pH 4.5)
sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (9)	4×10^{-6}
sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranoonate (11)	2.5×10^{-6} (N2 and N9 neuraminidase)
Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (12)	approx. 3×10^{-3}
Methyl 7,8,9-tri-O-acetyl-2,3,5-trideoxy-4',5'-dihydro-2'-methyl-oxazolo [5,4-d] D-glycero-D-talo-non-2-enopyranosonate (13)	3×10^{-5}
sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (14)	1×10^{-7} (N2 and N9 neuraminidase)
Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (17)	2.8×10^{-5}

Sodium 5-acetamido-4-N-methylamino-2,3,4.5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (20)	1.15×10^{-6}
Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (23)	7×10^{-7}
Methyl 5-acetamido-4-N-methoxycarbonylmethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (25)	7×10^{-6}
Sodium 5-acetamido-4-N-2'-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (29)	1.6×10^{-6} (N2 and N9 neuraminidase)
Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate (35)	4.8×10^{-4}
Sodium 4,5-Diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (38)	6.5×10^{-7}
Methyl 5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (41)	3.6×10^{-5}
5,9-diacetamido-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (42)	1.45×10^{-5}

Methyl 5-acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (43)	approx.	3	x 10^{-3}
5-Acetamido-9-cyano-2,3,5,9-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (44)		3	x 10^{-6}

Example 21 Inhibition of Influenza Virus Replication In Vitro

Inhibition of influenza A/Singapore/1/57 (H2N2) and Influenza B/Victoria/102/85 replication in vitro was measured by reduction of viral plaque formation in Madin Darby canine kidney (MDCK) cells

Monolayers of confluent MDCK cells, grown in six well tissue culture plates, were inoculated with 0.3 ml of virus diluted to give about 50-100 plaques/well. Virus was diluted in serum-free minimal essential medium (MEM) containing 2 µg/ml N-tosyl-1-phenylalanine chloromethyl ketone (TPCK) treated trypsin (Worthington Enzymes), and test compound.

Virus was adsorbed at room temperature for one hour, and the cells then overlaid with defined cell culture medium, version 1 (DCCM-1)/agar overlay containing test compound, 4 ml/well. DCCM-1 is a serum-free complete cell growth medium (Biological Industries), to which TPCK treated trypsin and DEAE-dextran to a final concentration of 2 µg/ml and 0.001% respectively, were added. Agar (5%) (Indubiose) was diluted 1:10 in the overlay before being added to the plate.

Once overlaid, plates were incubated at 37°C, 5% CO_2 for 3 days. Cells were then fixed with 5% glutaraldehyde, stained with carbol fuschin and the viral plaques counted. Results were as follows:

TABLE 2

Compound	IC_{50} Plaque Reduction (μM) Influenza A	Influenza B
Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6)	1.6	1.6
Sodium 5-Acetamido-4-amino-2,3,4,5-tetra deoxy-D-glycero-D-talo-non-2-enopyranosonate (14).	3.0	1.2
Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7)	1.6	1.6
Sodium 5-acetamido-4-N-2'-hydroxyethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (29)	60	7
Sodium 5-acetamido-4-N-allyl-N-hydroxy-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (45)	4.7	2.7
Sodium 4,5-Diamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (38)	11	6.8

Sodium 5-acetamido-4-N-allyl-N-hydroxy-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (45) can readily be prepared from compound (11) described in Example 5, using oxidation methods.

Example 22 In Vivo Anti-Viral Activity

The compounds of Examples 2,3 and 6 (4-amino, 4-guanidino and 4-epi-amino), as well as the compound DANA (2-deoxy-N-acetyl-α-D-neuraminic acid), which was shown in Example 20 to have anti-neuraminidase activity in vitro, were tested for anti-viral activity in a standard in vivo assay. When administered intranasally to mice before and during challenge with influenza A virus, these compounds reduced the titre of virus in lung tissue 1 to 3 days after infection.

Mice were infected intranasally with 50 µl of 10^3 $TCID_{50}$ units/mouse of H2N2 influenza A virus (A/Sing/1/57). The test compound was admionistered intranasally at a dose rate of either 12.5 or 25 mg/kg body weight (50 µl of aqueous solution/mouse) as follows: 24 hours and 3 hours before infection; 3 hours after infection then twice daily on each of days 1, 2 and 3 after infection. The structurally unrelated compounds ribavirin and amantadine were also used for comparison.

The mice were sacrificed on days 1, 2 and 3 after infection, their lungs removed and virus titres in the lungs measured. The titres were plotted graphically and expressed as the percentage area under the curves (AUC) compared to those for untreated mice. Results are summarized below.

Table 3

Efficacy in Influenza Virus Infected Mice

Experiment Number	Compound	Dose (mg/kg body weight)	% AUC
1	Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-amino-Neu5Ac2en) (6)	25	0.06
	Amantadine	25	0.08
	DANA	25	0.18
2	Ammonium 5-Acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (7)	12.5	0.03
	Ribavirin	25	29.8
	Amantadine	25	0.2
		12.5	0.03
	DANA	12.5	2.0
3	Sodium 5-Acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (14)	12.5	21.1
	Amantadine	12.5	8.8
	DANA	12.5	48.0

All three compounds tested showed greater potency than DANA.

Example 23 The following formulations are representative of compositions according to the invention:

AQUEOUS SOLUTION

	% w/w
Compound of formula (I)	10.0
Benzalkonium chloride	0.04
Phenylethyl alcohol	0.40
Purified water	to 100% w/w

AQUEOUS COSOLVENT SOLUTION

	% w/w
Compound of formula (I)	10.0
Benzalkonium chloride	0.04
Polyethylene glycol 400	10.0
Propylene glycol	30.0
Purified water	to 100% w/w

AEROSOL FORMULATION

	% w/w
Compound of formula (I)	7.5
Lecithin	0.4
Propellant 11	25.6
Propellant 12	66.5

DRY POWDER FORMULATION

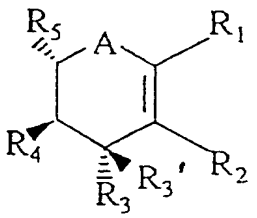
	% w.w
Compound of formula (I)	40.0
Lactose	60.0

These formulations are prepared by admixture of the active ingredient and excipients by conventional pharmaceutical methods.

It will be clearly understood that the invention in its general aspect is not limited to the specific details referred to hereinabove.

CLAIMS:

1. A compound of formula (I) or formula (Ia)



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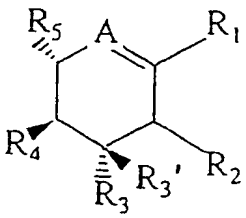


(Ia)

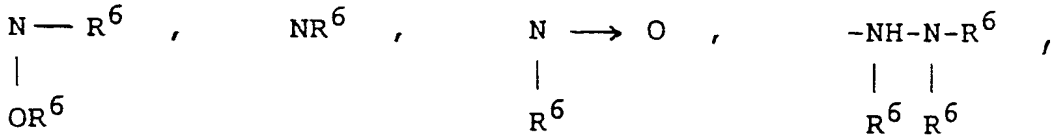
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

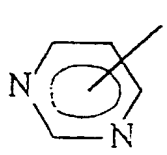
R^1 denotes COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, CHO or $CH(CHO)_2$,

R^2 denotes H, OR^6, F, Cl, Br, CN, NHR^6, SR^6 or CH_2X, wherein X is NHR^6, halogen or OR^6 and

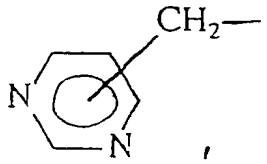
R^6 is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group,

R^3 and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, NHR^6, N_3, SR^6, $=N-OR^6$, OR^6, guanidino,





- or -



R^4 denotes NHR^6, SR^6, OR^6, $COOR^6$, NO_2, $C(R^6)_3$, CH_2COOR^6, CH_2NO_2 or CH_2NHR^6, and

R^5 denotes CH_2YR^6, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an R^5

group are the same or different,

and pharmaceutically acceptable salts or derivatives thereof,

provided that in general formula (I)

(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

(a) an R^2 that is hydrogen and

(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen, and that in general formula (Ia),

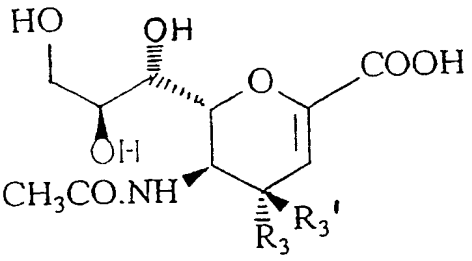
(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an R^2 that is hydrogen, and

(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen.

2. A compound as claimed in Claim 1 wherein the compound is a compound of formula (II)



(II)

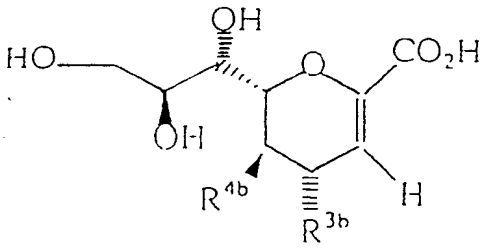
wherein R^3 is hydrogen or $R^{3'}$ and $R^{3'}$ is $-N_3$, $-CN$, $-CH_2NH_2$, or $-N.R^8.R^9$;

R^8 and R^9 are the same or different, and each denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substituted acyl group of 1 to 6 carbon atoms, $-C.(NH).NH_2$, $-CH_2.COOH$, CH_2-CH_2-OH or $-CH_2.CH.(R^{10})(R^{11})$,

R^{10} and R^{11} may be the same or different, and each denotes oxygen or $R^{12}N=$, and

R^{12} denotes hydrogen, $-OH$, $-OCH_3$, $-NH_2$, or $(CH_3)_2N-$or a pharmaceutically acceptable salt or derivative thereof.

3. A compound as claimed in Claim 1 or Claim 2 which is a compound of formula (Ib)



(Ib)

wherein R^{3b} is $(alk)_xNR^{6b}R^{7b}$, CN or N_3

where alk is unsubstituted or substituted methylene,

x is 0 or 1

R^{6b} is hydrogen, C_{1-6}alkyl, aryl, aralkyl, amidine, $NR^{7b}R^{8b}$ or an unsaturated or saturated ring containing one or more heteroatoms (such as nitrogen, oxygen or sulphur),

R^{7b} is hydrogen, C_{1-6}alkyl or allyl; or

$NR^{6b}R^{7b}$ forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms,

R^{8b} is hydrogen or C_{1-6}alkyl and

R^{4b} is $NHCOR^{9b}$ where R^{9b} is hydrogen, substituted or unsubstituted C_{1-4}alkyl or aryl,

or a pharmaceutically acceptable salt or derivative thereof.

4. A compound as claimed in any one of Claims 1 to 3 wherein R^3 is NHR^6.

5. A compound as claimed in Claim 3 wherein R^{3b} is $NR^{6b}R^{7b}$.

6. A compound as claimed in any one of Claims 1 to 5 wherein R^3 is

NH_2 or $NH\text{-}C(=NH)\text{-}NH_2$.

7. A compound as claimed in Claim 1 and selected from the group consisting of:-

Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-galacto-non-2-enopyranosonate (4-Azido-Neu5Ac2en);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-epi-amino-Neu4Ac2en);

Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-diallylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-allylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5,7,8,9$Ac_4$2en1Me);

8. 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

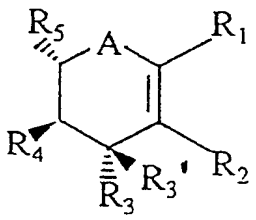
9. Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

10. 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

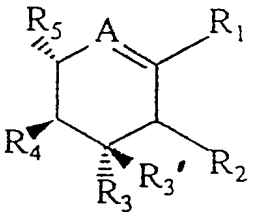
11. Ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

12. A pharmaceutical formulation comprising a compound

of formula (I) or formula (Ia)



(I)



(Ia)

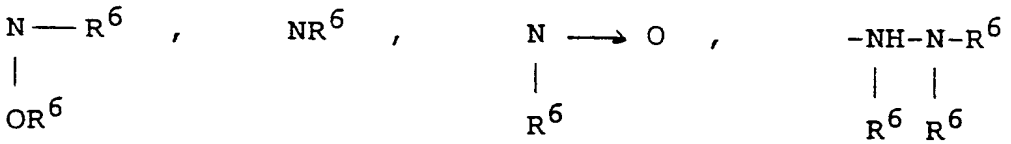
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

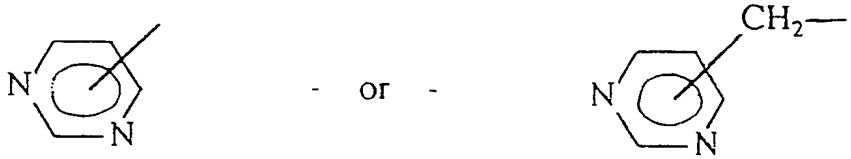
R^1 denotes COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, O or $CH(CHO)_2$,

R^2 denotes H, OR^6, F, Cl, Br, CN, NHR^6, SR^6 or CH_2X, wherein X is NHR^6, halogen or OR^6 and

R^6 is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group,

R^3 and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, NHR^6, N_3, SR^6, $=N-OR^6$, OR^6, guanidino,





R^4 denotes NHR^6, SR^6, OR^6, $COOR^6$, NO_2, $C(R^6)_3$, CH_2COOR^6, CH_2NO_2 or CH_2NHR^6, and

R^5 denotes CH_2YR^6, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an R^5 group are the same or different,

or a pharmaceutically acceptable salt or derivative thereof, together with a pharmaceutically acceptable carrier therefor.

13. A pharmaceutical formulation as claimed in Claim 12 wherein the compound of formula (I) is a compound as claimed in any one of Claims 1 to 9.

14. A pharmaceutical formulation as claimed in Claim 12 or Claim 13 wherein the formulation is adapted for intranasal administration.

15. Use of a compound of formula (I) as defined in Claim 12 in the manufacture of a medicament for the treatment of a viral infection.

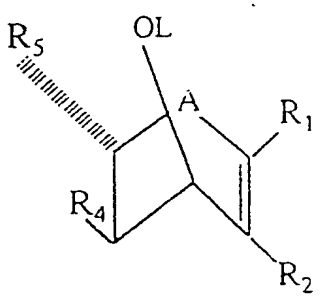
16. Use of a compound as claimed in Claim 15 wherein the viral infection is influenza.

17. A method for the treatment of a mammal including man suffering from a viral infection which comprises the step of administering to said mammal an effective amount of a compound of formula (I) as defined in Claim 11.

18. A method as claimed in Claim 17 wherein the viral infection is influenza.

19. A method for the preparation of a compound of formula (I) as defined in Claim 1 which comprises the steps of

(A) reaction of a compound of formula (III)



(III)

wherein R^1, R^2, R^4 and R^5 are as defined in Claim 1 and L is a leaving group with a nucleophile; or

(B) interconversion of one compound of formula (I) to another compound of formula (I), and if necessary or desired, subjecting the resulting compound to one or two further reactions comprising :

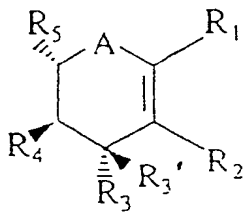
(i) removing any protecting groups;

(ii) converting a compound of formula (I) or a salt thereof into a pharmaceutically acceptable salt thereof.

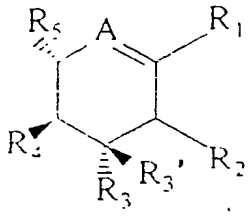
AMENDED CLAIMS

[received by the International Bureau
on 1 October 1991 (01.10.91):
original claim 13 cancelled:
original claims 1,3,7 and 12 amended;
new claims 10, 13-15, 19 and 24-37 added;
other claims unchanged (9 pages)]

1. A compound of formula (I) or formula (Ia)



(I)



(Ia)

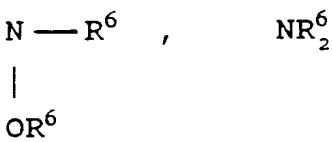
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

R^1 denotes COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, CHO or $CH(CHO)_2$,

R^2 denotes H, OR^6, F, Cl, Br, CN, NHR^6, SR^6 or CH_2X, wherein X is NHR^6, halogen or OR^6 and

R^6 is hydrogen; an acyl group having 1 to 4 carbon atoms; a linear or cyclic alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group,

R^3 and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, NHR^6, N_3, SR^6, $=N-OR^6$, OR^6, guanidino,



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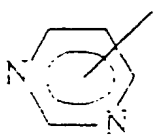
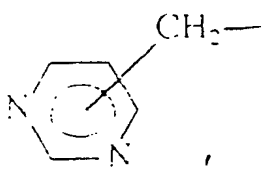


or



,

R^4 denotes NHR^6, SR^6, OR^6, $COOR^6$, NO_2, $C(R^6)_3$, CH_2COOR^6, CH_2NO_2 or CH_2NHR^6, and

R^5 denotes CH_2YR^6, $CHYR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an R^5

group are the same or different,

and pharmaceutically acceptable salts or derivatives thereof,

provided that in general formula (I)

(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is oxygen or sulphur, then said compound cannot have both

(a) an R^2 that is hydrogen and

(b) an R^4 that is O-acyl or NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen, and that in general formula (Ia),

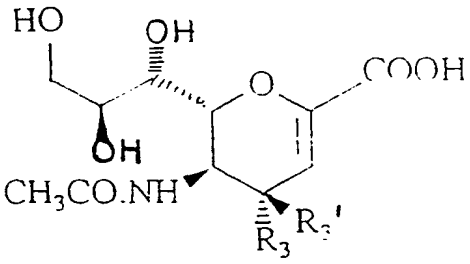
(i) when R^3 or $R^{3'}$ is OR^6 or hydrogen, and A is nitrogen, then said compound cannot have both

(a) an R^2 that is hydrogen, and

(b) an R^4 that is NH-acyl, and

(ii) R^6 represents a covalent bond when Y is hydrogen.

2. A compound as claimed in Claim 1 wherein the compound is a compound of formula (II)



(II)

wherein R^3 is hydrogen or $R^{3'}$ and $R^{3'}$ is $-N_3$, $-CN$, $-CH_2NH_2$, or $-N.R^8.R^9$;

R^8 and R^9 are the same or different, and each denotes hydrogen, a linear or cyclic alkyl group of 1 to 6 carbon atoms, an acyl or substituted acyl group of 1 to 6 carbon atoms, $-C.(NH).NH_2$, $-CH_2.COOH$, $-CH_2-CH_2-OH$ or $-CH_2.CH.(R^{10})(R^{11})$,

R^{10} and R^{11} may be the same or different, and each denotes oxygen or $R^{12}N=$, and

R^{12} denotes hydrogen, $-OH$, $-OCH_3$, $-NH_2$, or $(CH_3)_2N-$

or a pharmaceutically acceptable salt or derivative thereof.

3. A compound as claimed in Claim 1 or Claim 2 wherein R^3 is NHR^6.

4. A compound as claimed in any one of Claims 1 to 3 wherein R^3 is

NH_2 or $NH-\overset{\overset{NH}{\|}}{C}-NH_2$.

5. A compound as claimed in Claim 1 and selected from the group consisting of:-

Sodium 5-acetamido-4-azido-2,3,4,5-tetradeoxy-D-galacto-non-2-enopyranosonate (4-Azido-Neu5Ac2en);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate; (4-epi-amino-Neu4Ac2en);

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N,N-diallylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-diallylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-N-allylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate (4-N-allylamino-Neu5,7,8,9$Ac_4$2en1Me);

Sodium 5-acetamido-4-N,N-dimethylamino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate;

Sodium 2,-3-dideoxy-D-glycero-D-galacto-non-2-enopyranosonate; and

Methyl 5-acetamido-7,8,9-tri-O-acetyl-4-azido-2,3,4,5-tetradeoxy-D-glycero-D-talo-non-2-enopyranosonate (4-epi-azidoNeu5,7,8,9$Ac_4$2en1Me).

6. A pharmaceutical formulation comprising a compound of formula (I) or (Ia) as defined in any one of Claims 1 to 5, or a pharmaceutically acceptable salt or derivative

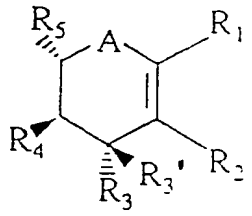
thereof, together with a pharmaceutically acceptable carrier therefor.

7. A pharmaceutical formulation as claimed in Claim 6 wherein the formulation is adapted for intranasal administration.

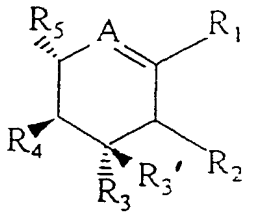
8. Use of a compound of formula (I) or (Ia) as defined in Claim 1 in the manufacture of a medicament for the treatment of a viral infection.

9. Use of a compound as claimed in Claim 8 wherein the viral infection is influenza.

10. A method for the treatment of a mammal including man suffering from a viral infection comprising administration of a compound of formula (I) or formula (Ia)



(I)



(Ia)

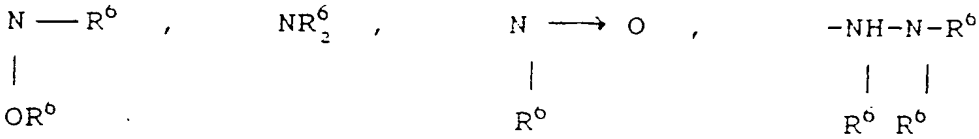
where in general formula (I), A is oxygen, carbon or sulphur, and in general formula (Ia), A is nitrogen or carbon;

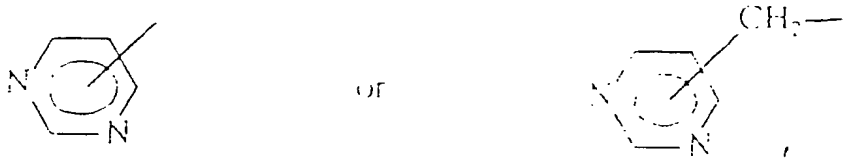
R^1 denotes COOH, $P(O)(OH)_2$, NO_2, SOOH, SO_3H, tetrazol, CH_2CHO, O or $CH(CHO)_2$,

R^2 denotes H, OR^6, F. Cl, Br, CN, NHR^6, SR^6 or CH_2X, wherein X is NHR^6, halogen or OR^6 and

R^6 is hydrogen; an accyl group having 1 to 4 carbon atoms; a linear or cyclicc alkyl group having 1 to 6 carbon atoms, or a halogen-substituted analogue thereof; an allyl group or an unsubstituted aryl group or an aryl substituted by a halogen, an OH group, an NO_2 group, an NH_2 group or a COOH group,

R^3 and $R^{3'}$ are the same or different, and each denotes hydrogen, CN, NHR^6, N_3, SR^6, $=N-OR^6$, OR^6, gaunidino,





R^4 denotes NHR^6, SR^6, OR^6, $COOR^6$, NO_2, $C(R^6)_3$, CH_2COOR^6, CH_2NO_2 or CH_2NHR^6, and

R^5 denotes CH_2YR^6, $CHR^6CH_2YR^6$ or $CHYR^6CHYR^6CH_2YR^6$, where Y is O, S, NH or H, and successive Y moieties in an R^5 group are the same or different,

or a pharmaceutically acceptable salt or derivative thereof.

11. A method for the treatment of a mammal including man suffering from a viral infection which comprises the step of administering to said mammal an effective amount of a compound of formula (I) or (Ia) as defined in Claim 1.

12. A method as claimed in Claim 11 wherein the viral infection is influenza.

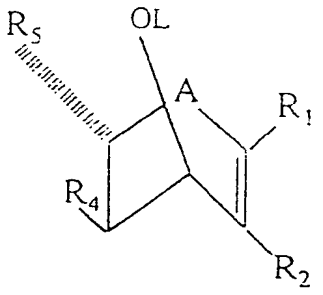
13. A method as claimed in either Claim 10 or Claim 11 wherein the infection is by a respiratory virus.

14. A method as claimed in any one of Claims 10 to 13 wherein the active ingredient is administered to the respiratory tract.

15. A method as claimed in any one of Claims 10 to 14 wherein the active ingredient is administered intranasally.

16. A method for the preparation of a compound of formula (I) as defined in Claim 1 which comprises the steps of

(A) reaction of a compound, of formula (III)



(III)

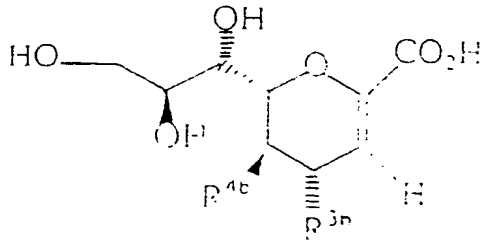
wherein R^1, R^2, R^4 and R^5 are as defined in Claim 1 and L is a leaving group, with a nucleophile; or

(B) interconversion of one compound of formula (I) to another compound of formula (I), and if necessary or desired, subjecting the resulting compound to one or two further reactions comprising :

(i) removing any protecting groups;

(ii) converting a compound of formula (I) or a salt thereof into a pharmaceutically acceptable salt thereof.

17. A compound of formula (Ib)



(Ib)

wherein R^{3b} is $(alk)_xNR^{6b}R^{7b}$, CN or N_3

where alk is unsubstituted or substituted methylene,

x is 0 or 1

R^{6b} is hydrogen, C_{1-6}alkyl, aryl, aralkyl, amidine, $NR^{7b}R^{8b}$ or an unsaturated or saturated ring containing one or more heteroatoms (such as nitrogen, oxygen or sulphur),

R^{7b} is hydrogen, C_{1-6}alkyl or allyl; or

$NR^{6b}R^{7b}$ forms an optionally substituted 5 or 6 membered ring optionally containing one or more additional heteroatoms,

R^{8b} is hydrogen or C_{1-6}alkyl and

R^{4b} is $NHCOR^{9b}$ where R^{9b} is hydrogen, substituted or unsubstituted C_{1-4}alkyl or aryl,

or a pharmaceutically acceptable salt or derivative thereof.

18. A compound as claimed in Claim 3 wherein R^{3b} is $NR^{6b}R^{7b}$.

19. A compound as claimed in Claim 17 or Claim 18 wherein R^{3b} is NH_2 or $NHC(=NH)NH_2$.

20. 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

21. Sodium 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

22. 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid and pharmaceutically acceptable salts and derivatives thereof.

23. Ammonium 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonate.

24. A pharmaceutical formulation comprising a compound as claimed in any one of Claims 17 to 23 as active ingredient together with a pharmaceutically acceptable carrier therefor.

25. A pharmaceutical formulation suitable for intranasal administration comprising a compound as claimed in any one of Claims 17 to 23 as active ingredient together with a pharmaceutically acceptable carrier therefor.

26. A pharmaceutical formulation as claimed in Claim 24 or Claim 25 wherein the active ingredient is 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

27. A pharmaceutical formulation as claimed in Claim 24 or Claim 25 wherein the active ingredient is 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

28. A method for the treatment of a mammal including man suffering from a viral infection comprising administration of an effective amount of a compound as

claimed in any one of Claims 17 to 23.

29. A method as claimed in Claim 28 wherein the infection is a viral respiratory infection.

30. A method as claimed in Calim 28 or Claim 29 wherein the viral infection is influenza.

31. A method as claimed in any one of Claims 28 to 30 wherein the active ingredient is administered to the respiratory tract.

32. A method as claimed in any one of Claims 28 to 31 wherein the compound is administered intranasally.

33. A method as claimed in any one of Claims 28 to 32 wherein the compound is 5-acetamido-4-amino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

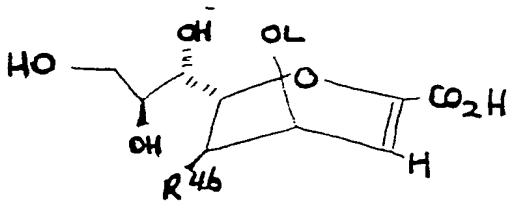
34. A method as claimed in any one of Claims 28 to 32 wherein the compound is 5-acetamido-4-guanidino-2,3,4,5-tetradeoxy-D-glycero-D-galacto-non-2-enopyranosonic acid or a pharmaceutically acceptable salt thereof.

35. Use of a compound as claimed in any one of Claims 17 to 23 in the manufacture of a medicament for the treatment of a viral infection.

36. A compound as claimed in any one of Claims 17 to 23 for use in medicine.

37. A method for the preparation of a compound of formula (Ib) as defined in any one of Claims 17 to 23 which comprises the steps of:

(A) reaction of a compound of formula (IIIb)



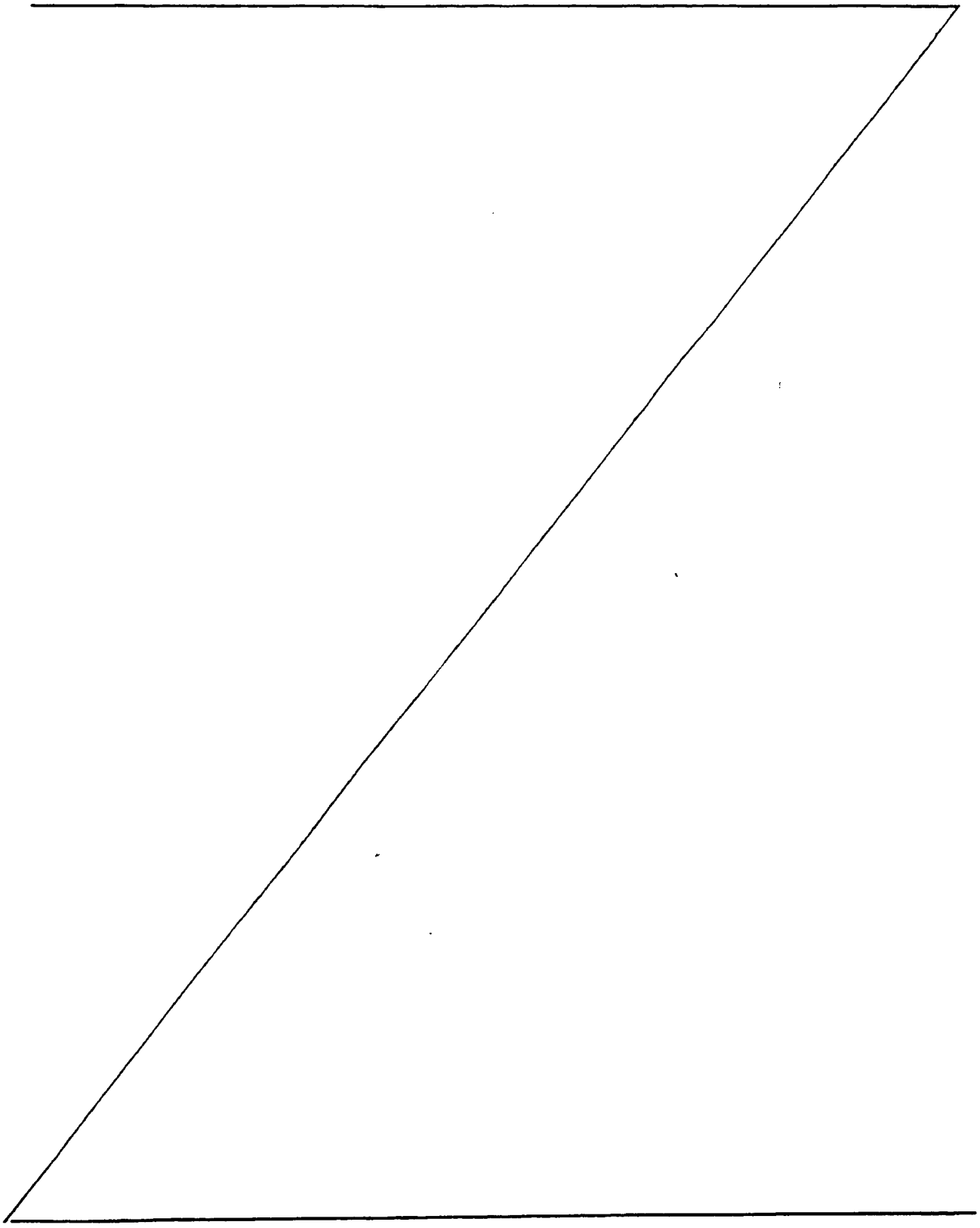
wherein R^{4b} is as defined in Claim 17 and L is a leaving group, or a protected derivative of said compound, with a nucleophile; or

(B) interconversion of one compound of formula (Ib) to another compound of formula (Ib)

and if necessary subjecting the resulting compound to one or two further reactions comprising:

(i) removing any protecting groups;

(ii) converting a compound of formula (Ib) or a salt thereof into a pharmaceutically acceptable salt thereof.



I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C07D 309/30; 309/28; 309/26; 309/22; C07D 309/20; 335/02; 211/78; 211/74; 211/72; 211/70

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
IPC	C07D 309/30; 309/28; 309/26; 309/22; 309/20; 335/02; 211/78; 211/74; 211/72; 211/70. Chemical Abstracts - ON-LINE" database for substructure search of pyran, thiopyran,piperidine and cyclohexane, with a glycerol moiety at position-2 optionally having a double bond at positions 5 or 6.

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

AU : IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
A	DE,A, 1493249 (Dr Karl Thomae GmbH) Claim 1	1-6
A	Tetrahedron Letters, vol. 28, No. 2, 1987, pages 191-194, published by Pergamon Journals Ltd. (U.K.) H. Mack and R. Brossmer, "Synthesis of 6-thiosialic acids and 6-thio-N-acetyl-D-neuraminic acid"	1-6
X	Chemical and Pharmaceutical Bulletin, vol. 36, no. 12, 1988, pages 4807-4813 published by Pharmaceutical Society of Japan M. Nakamura et al "Studies on sialic acids, XV, Synthesis of α , and β -O-glycosides of 3-deoxy-D-glycero-D-galacto-2-nonulopyranosonic acids (KDN)", page 4803, compound (13)	1

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report 2 August 91
International Searching Authority: **Australian Patent Office**	Signature of Authorized Officer: C.A. BRICK

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:
NIL

2.[] Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
NIL

3.[] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a): NIL

VI. [] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:
NIL

1. [] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest
[] The additional search fees were accompanied by applicant's protest.
[] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 91/00161

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members
DE 1439249	

END OF ANNEX

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 August 1999 (27.08.99)	
International application No. PCT/US98/26327	**Applicant's or agent's file reference** 237.F
International filing date (day/month/year) 10 December 1998 (10.12.98)	**Priority date** (day/month/year) 12 December 1997 (12.12.97)
Applicant KIM, Choung, U. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 June 1999 (16.06.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

F. Baechler

Telephone No.: (41-22) 338.83.38

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C07C 233/52, 69/75, 69/757, A61K 31/16, 31/215

A1

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(22) International Filing Date: 10 December 1998 (10.12.98)

(30) Priority Data:
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(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/069,553 (CON)
Filed on 12 December 1997 (12.12.97)

(71) Applicant *(for all designated States except US)*: GILEAD SCIENCES, INC. [US/US]; 333 Lakeside Drive, Foster City, CA 94404 (US).

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(74) Agents: BOSSE, Mark, L. et al.; Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404 (US).

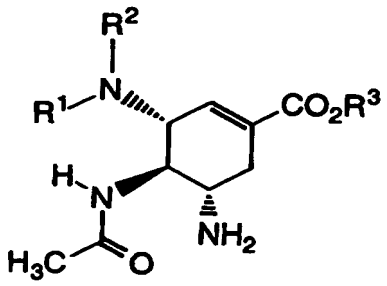
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CYCLOHEXENE CARBOXYLATES AS NEURAMINIDASE INHIBITORS

(57) Abstract

Novel compounds of Formula (I) are described. R^1, R^2, R^3, R^4, R^5 and R^6 are described in this specification. Synthetic intermediates and pharmaceutical compositions comprising the inhibitors of the invention are also described. Methods of inhibiting neuraminidase in samples suspected of containing neuraminidase are also described. Assay methods for detecting neuraminidase activity are also described.



(I)

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/26327

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C233/52 C07C69/75 C07C69/757 A61K31/16 A61K31/215

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 16320 A (BIOTA SCIENT MANAGEMENT) 31 October 1991 cited in the application	1

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 1999	07.04.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/26327

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 40-42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/26327

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9116320 A	31-10-1991	AP 249 A	17-03-1993
		AT 161253 T	15-01-1998
		AU 7533891 A	12-12-1991
		AU 654815 B	24-11-1994
		AU 7759091 A	11-11-1991
		CA 2081356 A	25-10-1991
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		DE 69128469 D	29-01-1998
		DE 69128469 T	04-06-1998
		EP 0526543 A	10-02-1993
		EP 0786458 A	30-07-1997
		ES 2113881 T	16-05-1998
		FI 924790 A	22-10-1992
		GR 3026225 T	29-05-1998
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		HU 9500070 A	28-04-1995
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		PT 97460 A	31-01-1992
		SG 43170 A	17-10-1997
		SI 9110745 A	30-04-1997
		US 5648379 A	15-07-1997
		US 5360817 A	01-11-1994

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
237.F		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US98/26327	10/12/1998	12/12/1997
International Patent Classification (IPC) or national classification and IPC C07C233/52		
Applicant GILEAD SCIENCES, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/06/1999	10.09.99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Goetz, G Telephone No. +49 89 2399 8105

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/26327

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

   Description, pages:

   1-33 as originally filed

   Claims, No.:

   1-42 as originally filed

2. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 40-42.

because:

☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-42
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-42
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-39
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

For the assessment of the present claims 40-42 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 40-42 relate to subject matter considered by this Authority to be overed by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject matter of this claim (Article 34(4)(a)(i) PCT)

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The prior art (WO-A-9116320, cited) does not disclose any compound according to one of the formulae claimed in present claims 1 to 39.
   The subject matter of claims 1 to 42 is thus regarded to be novel over said prior art (Article 33.2 PCT).

2. The claimed compounds differ in a non-obvious way from the structure of the prior art compounds of D1 (which are also inhibitors of neuramidase) that they are considered to be alternatives involving an inventive step (Article 33.3 PCT).
   The subject matter of claims 1 to 42 is thus regarded involve an inventive step over said prior art (Article 33.2 PCT).

3. The industrial applicability can also be acknowledged for all examined claims (Article 33.4 PCT).

Re Item VII

Certain defects in the international application

1. The meaning for "-OMs" in claims 33, 34 and 37 in missing. The definition as given on page 9 should be included in these claims.

2. The statement on present page 33 contains not only irrelevant subject matter (Rule 9.1iv PCT) but leads also to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).